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NICHOLAS HANSEN LOW

TITLE OF THESIS

A GENERAL 2',3'-UNSATURATION PROCEDURE FOR NUCLEOSIDES

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THE UNIVERSITY OF ALBERTA

A GENERAL 2',3'-UNSATURATION PROCEDURE FOR NUCLEOSIDES

by

NICHOLAS HANSEN LOW

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

SPRING 1984

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled A GENERAL 2',3'-UNSATURATION PROCEDURE FOR NUCLEOSIDES submitted by NICHOLAS HANSEN LOW in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE.

Abstract

A general and efficient procedure for the preparation of 2',3'-unsaturated nucleosides has been developed. Treatment of the parent ribonucleosides with α-acetoxy-isobutyryl bromide gave the 2'-O-acetyl-3'-bromo-3'-deoxy xylo (and as the minor isomer 3'-O-acetyl-2'-bromo-2'-deoxy arabino) 5'-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl) substituted nucleosides. In some cases a minor amount of the corresponding nucleoside derivative with a free 5'-hydroxyl function was observed. In such cases this was protected by acetylation prior to conversion to the 2',3'-unsaturated nucleoside. Reductive elimination of bromide and acetate from these compounds was effected using a zinc-copper couple in dimethylformamide. Deprotection with concomitant removal of residual zinc and copper was effected by chromatography on Dowex 1x2 (OH^-) resin. Further purification when necessary utilized chromatography on neutral silica gel. Good yields of the 2',3'-dideoxy-2',3'-didehydro nucleosides derived form adenosine, tubercidin, inosine, toyocamycin, sangivamycin, cytidine, uridine and guanosine were realized using this sequence.

Proton nuclear magnetic resonance spectra of the 2'-ene compounds have H2' and H3' coupling values of approximately 6Hz. The signal for H2' was found to be upfield from that for H3'. Correction of this long-standing misassignment of

signals in the literature was verified by deuterium substitution. The ^{13}C spectra of these compunds, in harmony with the proton data, showed the C2' carbon signal to be upfield from that of C3'. This assignment was also verified by deuteration at C3'.

The present work also provides convenient access by hydrogenation of the 2',3'-unsaturated products to the 2',3'-dideoxynucleosides that are used as DNA chain terminators in sequencing studies. High-yield conversion of adenosine to its 2',3'-anhydro and 2'- and 3'-deoxy derivatives have been developed also.

Acknowledgments

I am grateful to the faculty members, support staff, nonacademic staff and my colleagues in the Chemistry Department who have been of great aid during this thesis project. I am grateful to Dr. M.J. Robins for providing a laboratory area and considerable help during this work. Also, to Dr. F. Hansske for his helpful suggestions and ideas.

I would especially like to thank my parents for providing an opportunity to complete this work and for their enthusiastic encouragement.

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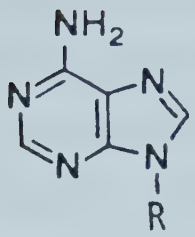
A. Introduction

In 1871 Miescher[1] isolated a material from pus cells that he called nuclein. Altmann[2] first introduced the term "nucleic acid" in 1889 when he successfully isolated these materials from a number of sources. In 1891, Kossel[3] reported the first results of hydrolyses of nucleic acids. This led to the identification and synthesis of the five major naturally occurring heterocyclic bases by Kossel, Fischer, Traube and Steudel.[4] These are, in the case of ribonucleic acid (RNA), the purines adenine (1) and guanine (2) and the pyrimidines cytosine (3) and uracil (5). In deoxyribonucleic acid (DNA) the same major bases occur with the exception of uracil, which is replaced by thymine (4). In addition to these five bases, over thirty others have been found to be present in nucleic acid in minor amounts. Most of the modified bases are methylated derivatives.[5]

In 1909 the term "nucleoside" was used by Levene and Jacobs[6] to describe the carbohydrate derivatives of purines and pyrimidines which had been isolated from alkaline hydrolyses of yeast nucleic acid. It has since been extended to include any carbohydrate derivative linked through the C-1 carbon to a heterocyclic base, whether by a C-N or a C-C bond.

Kossel suggested that acidic hydrolysis of RNA liberated a carbohydrate derivative. Levine and Jacobs[7]



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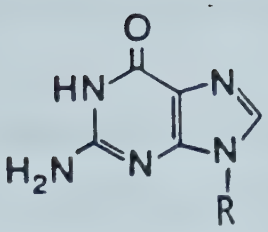
6 R = R′
10 R = R″



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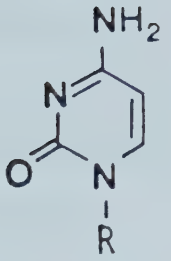

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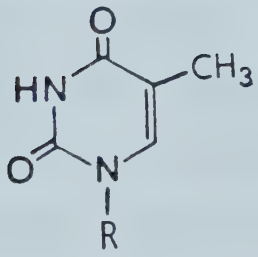

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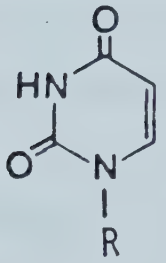

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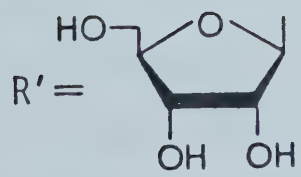
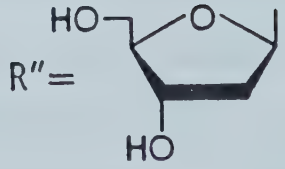


RIBOSE



2-DEOXY-D-RIBOSE

correctly characterized the carbohydrate moiety as D-ribose. Later, Levine and Mori[8] identified the sugar present in DNA as 2-deoxy-D-ribose. Levine and Tipson[9] subsequently determined the furanosyl structure of the ribose moiety by methylation studies.

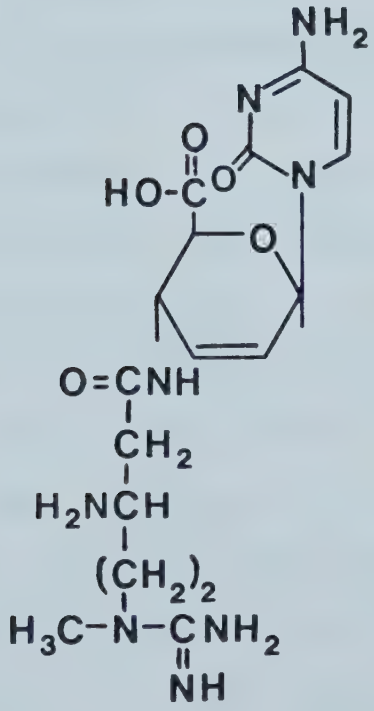
The position of attachment of the sugar moiety to the heterocyclic base was assigned by ultraviolet spectral comparisons.[10-12] Further confirmation by Todd and coworkers[13] was accomplished by comparisons of the periodate oxidation products of synthetic and natural compounds.

Todd and coworkers[14] also determined the β-configuration of nucleosides by demonstration of the formation of cis-intramolecularly linked 5'-cyclonucleosides derived from cytidine (8) and adenosine (6). Total syntheses of the two major purine ribonucleosides was achieved by Todd's group as the final structure proof.[15] Historical development of the identification and characterization of the nucleosides has been reviewed extensively.[16-21]

Thus the commonly occurring nucleosides found in RNA are 9-(β-D-ribofuranosyl)adenine (adenosine, 6), 9-(β-D-ribofuranosyl)guanine (guanosine, 7), 1-(β-D-ribofuranosyl)-uracil (uridine, 9) and 1-(β-D-ribofuranosyl)cytosine (cytidine, 8). In DNA they are 9-(2-deoxy-β-D-erythro-pento furanosyl)adenine (2'-deoxyadenosine, 10), 9-(2-deoxy-β-D-erythro-pentofuranosyl)guanine (2'-deoxyguanosine, 11), 1-(2-deoxy-β-D-erythro-pentofuranosyl)cytosine (2'-deoxycytidine, 12), and 1-(2-deoxy-β-D-

erythro-pentofuranosyl)thymine (2'-deoxythymidine, 13).

The first example of an unsaturated nucleoside was found in the antibiotic Blasticidin S by Misato and coworkers.[22] This antibiotic was found to inhibit the virulent fungus, *Piricularia oryzae* , the microorganism responsible for a rice plant disease.[23] Fox and coworkers[24] proposed a 2',3'-unsaturated hexopyranosylcytosine moiety in Blasticidin S. Confirmation of the 2',3'-ene structure was obtained by oxidation and other degradative studies. Blasticidin S (14) has also been found to be an inhibitor of protein synthesis in *E. coli*.[25-26]



Blasticidin S (14)

Related pyranosyl 2',3'-dideoxynucleoside antibiotics include the pentopyranines A and B, amicetins A and C, and pliacetin.

The DNA in the nuclei of eukaryotic cells contains all the information necessary for the biosynthetic pathways and metabolism of the cell. Through the specific ordering of the nucleic acid units in these macromolecules, a unique code is established. This code is transcribed from the parent to daughter DNA molecules during replication and cell division. The 2',3'-dideoxynucleosides, as their 5'-triphosphate esters, inhibit biosynthesis of DNA by acting as polynucleotide chain terminators (resulting from the absence of the "natural" 3'-hydroxyl group).[27]

The sequence of nucleotides in a cell's DNA codes for the primary sequence of all that cell's proteins. It also contains the information for regulation of the synthesis of proteins for enzymes and immunological defense mechanisms of the cell. Thus, the determination of DNA sequences is of major importance for the investigation of cellular biology.

Even the smallest phage DNA's, ØX174 and SV40, are approximately 5500 nucleotides or base pairs long, nearly 100 times longer than the longest RNA sequence which has been determined.[28] The DNA's need to be cleaved to defined fragments of about 50 to 100 nucleotides, about tRNA size, for convenient sequence analysis. By using endonucleases that cleave at specific sequences, large DNA molecules can be broken down first to "ØX size" and further to still

smaller fragments. The RI restriction enzyme of *E. coli* is a specific endonuclease that cleaves a DNA duplex to "ØX size" by introducing two single-strand, staggered breaks in a specific hexanucleotide sequence.[29]

In addition to the use of specific endonucleases, there are other approaches to obtaining defined DNA fragments.[30-39] One of these methods known as the chain terminator sequencing procedure makes use of 2',3'-dideoxynucleosides.

All of these methods rely on high resolution electrophoresis on denaturing polyacrylamide gels to resolve oligonucleotides with one common end but varying in length at the other by a single nucleotide. The chain terminator sequencing procedure of Sanger et al[40] is considered to be the most simple, rapid and accurate of this kind of sequencing methods.

The chain terminator method makes use of the 2',3'-dideoxy and the β-D-arabinofuranosyl analogs of the deoxyribonucleoside triphosphates and their incorporation by DNA polymerase I onto the 3'-hydroxyl of an extending transcript.[27]

Atkinson *et al.*[27] showed that the rate of incorporation of these analogs onto the 3' end (about 10^{-3} the rate of incorporation of a deoxyribonucleoside monophosphate) is also reflected in their correspondingly slower rate of removal by the 3'-5' hydrolytic and pyrophosphorylytic activities of DNA polymerase I. Effectively then, a 3' end

terminated by a dideoxynucleoside monophosphate is inert to further extension (even by removal of the terminating analogs by the proof reading function of the polymerase).[41]

The chain terminator method involves synthesis by the Klenow subfragment of DNA polymerase I[42] (which lacks the 5'→3'-exonuclease activity of the intact enzyme) of a complementary copy of the single-stranded target sequence, primed with the directly adjacent annealed strand of a restriction fragment. The synthesis is carried out in the presence of the four deoxyribonucleoside triphosphates, one or more of which is α-^{32}P-labelled , using each of the 2',3'-dideoxynucleoside triphosphate analogs in separate incubations. There is, therefore, in each reaction a base specific <u>partial</u> incorporation of a terminating analog onto the 3' ends of the extending transcripts throughout the sequence. Parallel fractionation (by gel electrophoresis) of the size ranges of terminated labelled transcripts from each reaction, each with the common 5' end of the primer, allows a sequence to be deduced.[43] As an alternative to the 'Klenow DNA polymerase I', reverse transcriptase[44] can be used in this procedure. This similarly incorporates dideoxynucleoside triphosphates leading to chain termination, although the reaction conditions required are slightly different.

The chain termination sequencing method has a number of advantages over the plus and minus[45] and partial ribosubstitution[46] procedures. It is more rapid since it involves a single step reaction. There is a higher

incorporation of counts from the ^{32}P-labelled triphosphates since the reaction can be carried out for a long enough period of time to allow extension from every primer. Most importantly, each sequential nucleotide produces a radioactive band, even with repetitive sequences of the same nucleotide.

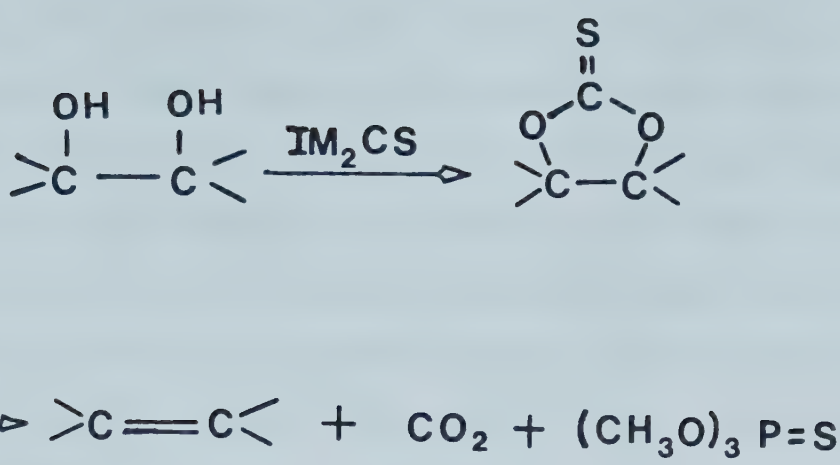
This technique has been applied to the DNA of many bacteriophages, a few examples are ØX174[40], SV40 DNA,[28] and λDNA.[28]

B. Conversion of Diols to Alkenes

Conversion of cis 1,2-diols to the corresponding alkenes has been studied by several groups. The Corey-Winter reaction was reported in 1963.[47] This reaction is useful for stereospecific synthesis since it allows the control of both the stereochemistry and the position of unsaturation. This method involves conversion of the 1,2-diol to a cyclic thionocarbonate (by the use of thiocarbonyl diimidazole in toluene or xylene at reflux) derivative which is transformed into the olefin by treatment with either trimethyl- or triethylphosphite.

The elimination process which comprises the second step of this olefin synthesis is postulated to involve a carbene intermediate which is unstable relative to olefin and carbon dioxide. (scheme A)

Josan and Eastwood[48] found that phenyl-substituted 2-ethoxy-1,3-dioxolans (orthoformates) undergo a cis-elimination reaction with loss of carbon dioxide and ethanol when heated in the presence of benzoic acid. Several other "reductive elimination" reactions have been described that allow the conversion of a 1,2-diol into the corresponding olefin by fragmentation of an intermediate 1,3-dioxolan derivative. These reactions include the carboxylic acid catalysed thermal decomposition of 2-alkoxy-1,3-dioxolans[49], the pyrolysis of the cyclic ketals of substituted



SCHEME A

norbornadienones[50] and the pyrolysis of derivatives of 1,3,6,9-tetraoxaspiro-4,4-nonane formed by the reaction of hexafluoroacetone with 2-methoxy-1,3-dioxolans.[51]

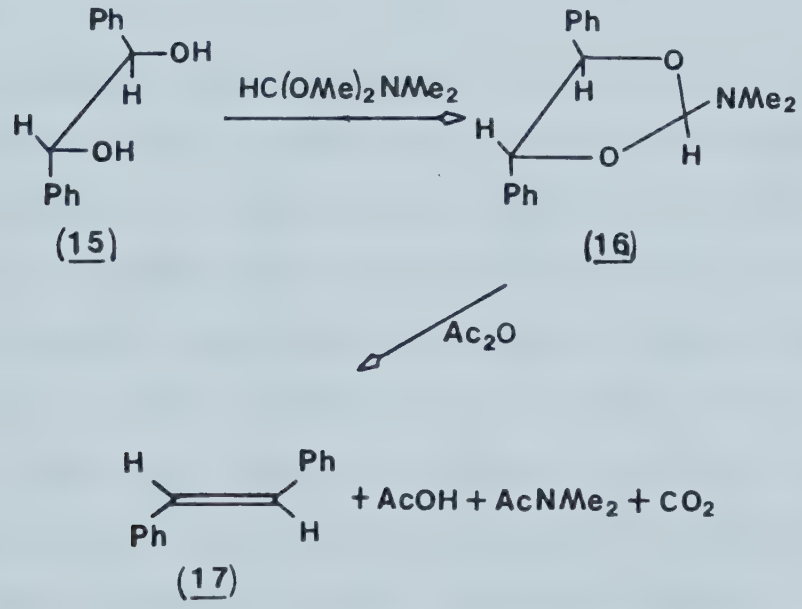
Various metal complexes also have been used to bring about this reductive elimination.[52-53] Whitman *et al.*[54-56] found that treatment of 2-phenyl-1,3-dioxolans with n-butyl-lithium gave olefins in a highly stereospecific fragmentation reaction in favourable cases. These authors reacted the benzylidene derivative of trans-cyclooctane-1,2-diol with n-butyl-lithium and obtained a 75% yield of trans-cyclooctene. The stereospecificity of this fragmentation was emphasized by the formation of cis-cyclooctene from either of the diastereoisomers of the benzylidene derivative of cis-cyclooctane-1,2-diol.

It was found, however, that this dioxolan-based olefin synthesis was not applicable to the synthesis of conjugated aryl-substituted olefins. The presence of the aryl group at C-4 or C-5 in the dioxolan facilitates proton abstraction from the α-aryl site. The authors suggested that the greater stability of a carbanion adjacent to one, rather than two, oxygen atoms is the factor that leads to that predominant mode of reaction.[57]

In 1965 Tipson and Cohen[58] reported the reinvestigation of a reaction previously described by Tipson and coworkers.[59] They found that terminal unsaturation could be introduced into an alditol by the action of sodium iodide (in a suitable solvent) on a derivative having contiguous primary and secondary sulfonyloxy groups.[59] In their earlier work, the authors had noted that vicinal secondary sulfonates such as the 3,4-dimethanesulfonates and 3,4-di-p-toluenesulfonate of 1,2:5,6-di-O-isopropylidene-D-mannitol failed to give unsaturated compounds under the usual conditions.

They reinvestigated these reactions and found that elimination occurred when various high boiling solvents (such as 2,5-hexanedione) were used. Free iodine and the sodium methanesulfonate-iodide salt were formed, and subsequent iodination occurred.[60-61] Use of sodium iodide/N,N-dimethylformamide/zinc dust at reflux resulted in elimination and reductive removal of iodine.

In 1970 Eastwood *et al.*[42] reported that treatment of vicinal diols, such as racemic 1,2-diphenylethane-1,2-diol (15), with N,N-dimethylformamide dimethyl acetal at elevated temperatures gave 2-dimethylamino-trans-4,5-diphenyl-1,3-dioxolan (16). Reaction of this dioxolan with acetic anhydride at 180°C yielded the corresponding trans alkene, trans-diphenylethylene (17), plus acetic acid, N,N-dimethyl-acetamide and carbon dioxide. (scheme B)



(15) (16) (17)

SCHEME B

These authors applied this method to more complex molecules with resulting yields in the 80-90% range.[42] The mechanism of this elimination reaction was not determined, but it was suggested that it could be similar to that operating in the acid-catalysed fragmentation of 2-ethoxy-1,3- dioxolans.[43-45] The reaction appeared to be

stereospecific, but the resulting alkene may undergo acid-catalysed isomerisation.

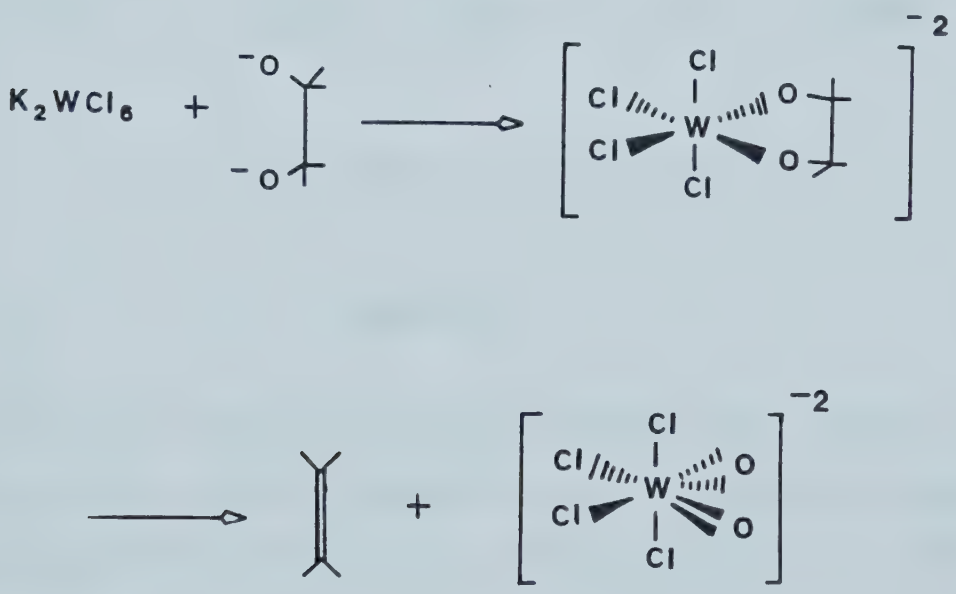
In 1972 Carnahan and Closson[43] noted that vicinal diol dimethanesulfonates undergo a rapid and high yield conversion to the corresponding alkene upon treatment with sodium anthracene or naphthalene (in THF or dimethoxyethane). They found that the reaction was non-stereospecific and the more stable alkene predominated. They also found that no migration of the double bond occurred in the cases studied.

This method has advantages relative to the reaction of the dimesylate with iodide ion[58], phosphite treatment of the thione carbonate[47], pyrolysis of cyclic orthoesters[49], and treatment of benzaldehyde acetals with butyl lithium.[54] Each of these methods requires long reaction times, high temperatures, and/or formation of a cyclic derivative from the diol. The anion radical technique, however, suffers from the disadvantage of reducing many other functional groups, for example, carbonyl, nitro, cyano and halogen.

Several other reducing agents were examined for their ability to convert dimesylates to alkenes. Treatment of cis-1,2-cyclooctanediol dimesylate with sodium in liquid ammonia afforded a 27% yield of cis-cyclooctene, and sodium in trimesitylborane[45] gave a 62% yield of the alkene.[43] Zinc dust in boiling acetic acid did not give the alkene. The authors suggested that the mechanism of elimination presumably involves electron transfer to one of the mesylate

groups, followed by C-O cleavage as in mono-mesylates.[6] Further reduction of the alkyl radical to the carbanion[7] followed by elimination of the adjacent mesylate as methanesulfonate anion completes the sequence.

Sharpless and Flood[8] devised a vicinal diol to olefin transformation based on reversal of the process involved in the reaction of permanganate or osmium tetroxide with an olefin. Their reaction scheme involves treatment of a vicinal dialkoxide with a tungsten (IV) halide derivative in refluxing tetrahydrofuran. (scheme C)

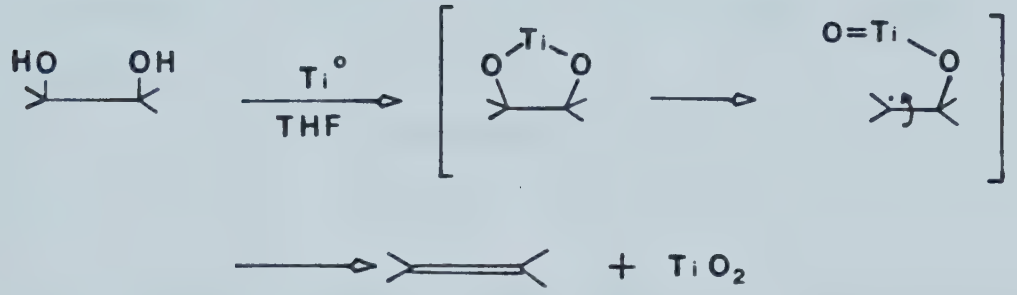


SCHEME C

Their data indicate that the olefin is formed by a syn-elimination. Some isomeric olefin was observed, but in a ratio that paralleled the composition of the starting diol. This transformation can be performed in a single reaction

vessel and is particularly useful for the preparation of tetrasubstituted olefins.

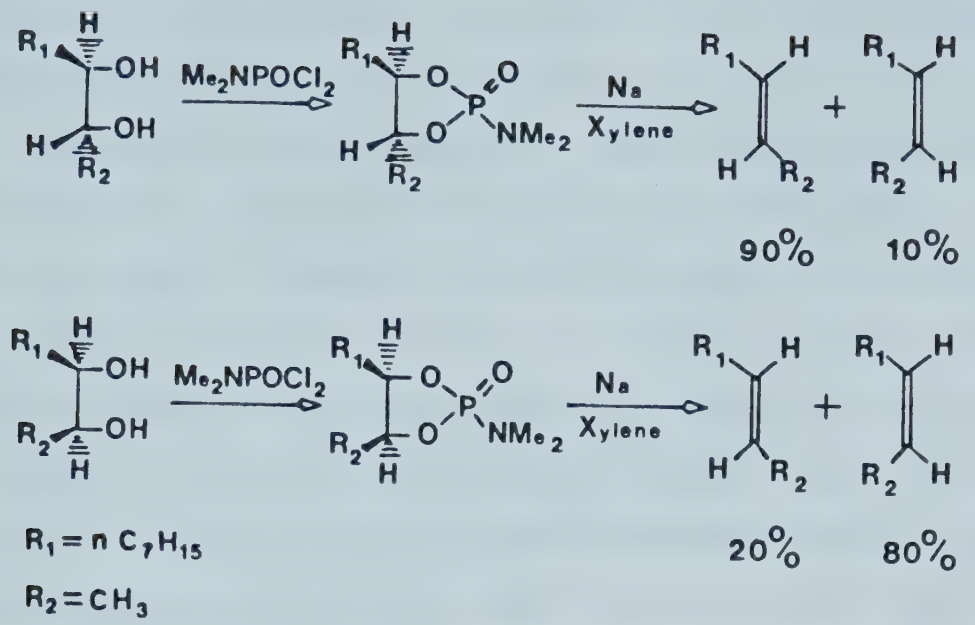
McMurry and Fleming[9] have shown that an active Ti metal powder, prepared by Rieke's[10] general method, can reduce 1,2-diols directly to olefins. They proposed that the reaction mechanism involves a five-membered ring intermediate which collapses in a nonconcerted manner. (scheme D)



SCHEME D

Both the meso and dl diols from 5-decene were reduced in good yield (75 and 80% respectively), but neither reaction is stereospecific (60:40 trans/cis and 90:10 trans/cis, respectively).

Marshall and Lewellyn[11] reported that vicinal diols react with N,N-dimethylamidophosphorodichloridate to yield cyclic amidophosphate esters. They prepared these derivatives of threo- and erythro-2,3-decanediol and subjected them to reaction with sodium in refluxing toluene. Both geometric alkene isomers were formed indicating that the reaction is non-stereospecific. (scheme E)

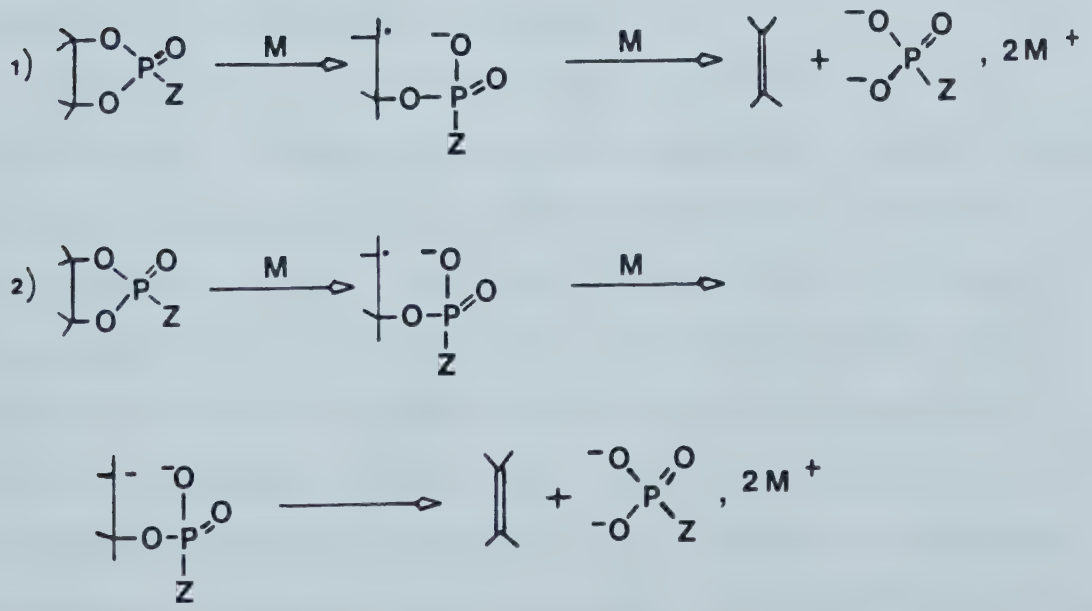


SCHEME E

In a further study by these workers[72], examination of cyclic ethyl phosphate derivatives was undertaken with a survey of reducing agents. This was prompted by the work of Ireland *et al.*[73] who found that tertiary alcohols are more reactive with diethyl phosphorochloridate than with bis-(dimethylamino)phosphorochloridate. Treatment of trans-1,2-dimethyl-1,2-cyclododecanediol with ethyl- or N,N-dimethylamidophosphorodichloridate followed by reduction of the resulting cyclic phosphate ester derivatives with lithium in ammonia afforded an 81:19 mixture of trans- and cis-1,2-dimethylcyclododecene, respectively from the ethyl and a 92:8 trans/cis mixture from the dimethylamido intermediates. It was found that the titanium reagents of McMurry[74] effected the reduction-elimination of cyclic

phosphate esters as efficiently as the alkali metal reagents. Reducing agents such as zinc, aluminum amalgam and chromium perchlorate were ineffective.

According to these authors, the reductive-elimination mechanism is a two-step process and they offer two possibilities.[72] (scheme F) In either case, rotation about the carbon-carbon bond of the intermediate radical anion (or dianion) leads to the intermediate product of net anti elimination. The syn-anti ratio will depend upon the relative timing of the first and second bond breaking steps, and possibly the relative transition state stability for phosphate explusion in a carbanion-type elimination reaction.

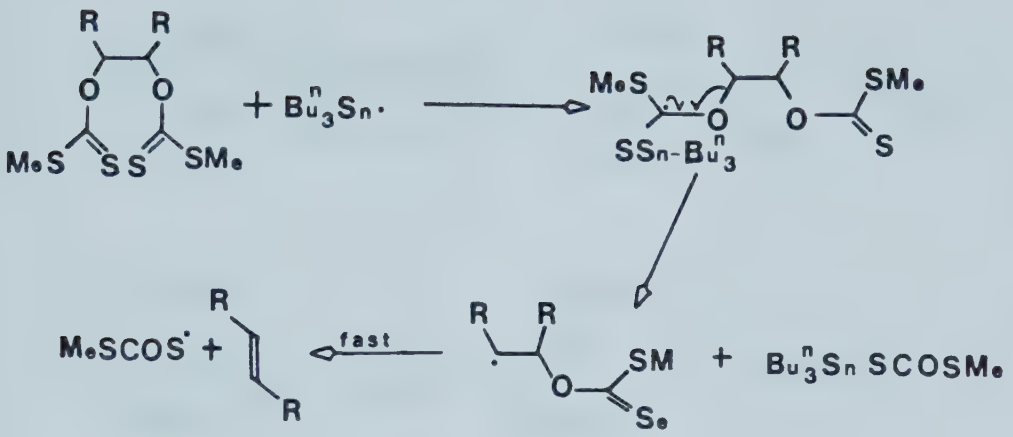


SCHEME F

In 1979 Barton *et al.*[74] reported a synthesis of alkenes from vicinal diols based on the reaction of bisdithiocarbonates with tri-n-butylstannane. The preparation of the bisdithiocarbonates involved the treatment of vicinal diols with sodium hydride and imidazole followed by addition of carbon disulfide and then methyl iodide as developed earlier.[75]

Heating the bisdithiocarbonates with tri-n-butylstannane in toluene at reflux gave the corresponding olefins in good yields. The (meso)- and dl-hydrobenzoin bis-xanthates were prepared and subjected to this reaction with tri-n-butylstannane. Only (E)-stilbene was observed in both cases. The authors concluded that the preference for E stereochemistry and the formation of the alkene from both bis-xanthates were consitent with the stepwise radical fragmentation presented in scheme G.[74]

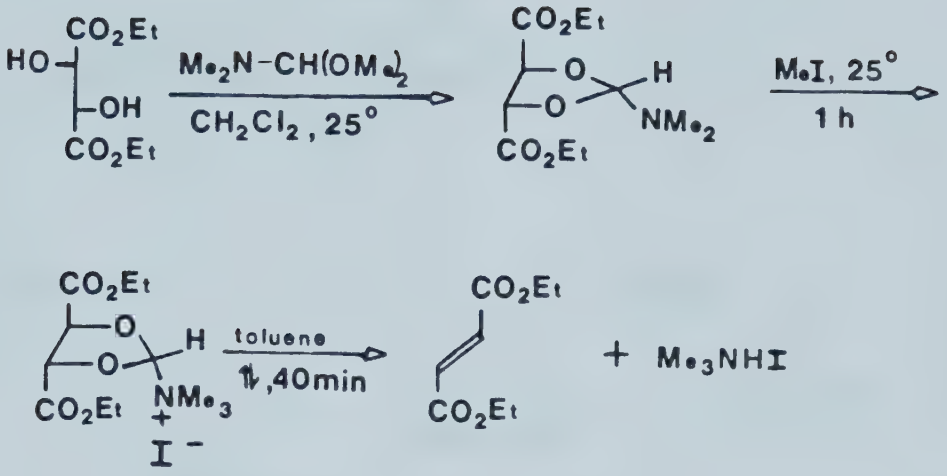
Hanessian *et al.*[76] described a procedure for the conversion of vicinal cis-diols in acyclic or cyclic systems (and vicinal trans-diols in acyclic systems) into the corresponding olefins. This two-stage, one-pot process is illustrated by the preparation of diethyl fumarate from diethyl D-tartrate.(scheme H) Their reaction sequence involved conversion of the diol into the corresponding 1-dimethylamino(methylene)acetal, by treatment with excess N,N-dimethylformamide dimethylacetal. The cyclic acetal was then treated with excess methyl iodide and a suspension of the resulting trimethylalkylammonium salt was heated at



SCHEME G

reflux in toluene to give the olefin.

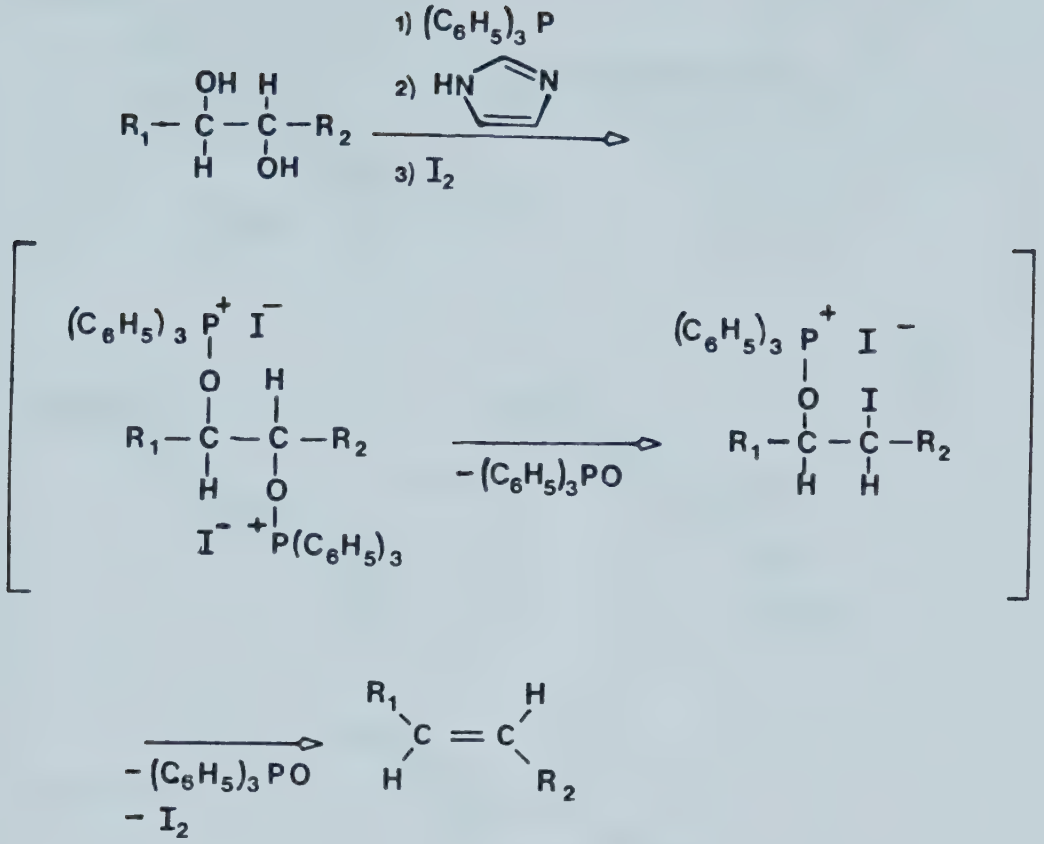
In 1979, Garegg and Samuelsson[77] reported a method for the conversion of vicinal diols to olefins involving a reagent system composed of triphenylphosphine, imidazole and iodine. (scheme I) Their method is useful for conversion of trans-1,2-diols into the corresponding olefins. This reaction sequence involves addition of iodine to a refluxing solution of the diol, triphenylphosphine and imidazole in toluene. The authors suggest that imidazole performs the dual function of a base plus forming a partially solvated complex with triphenylphosphine and iodine. Conversions of carbohydrate diols to olefins were effected in yields ranging from 29-59%. This method is ineffective for the conversion of cis-1,2-diols into olefins unless one



SCHEME H

equivalent of tetrabutylammonium iodide/per mole of diol and excess potassium iodide were added. Even with these additions, yields are poor (approximately 30%).

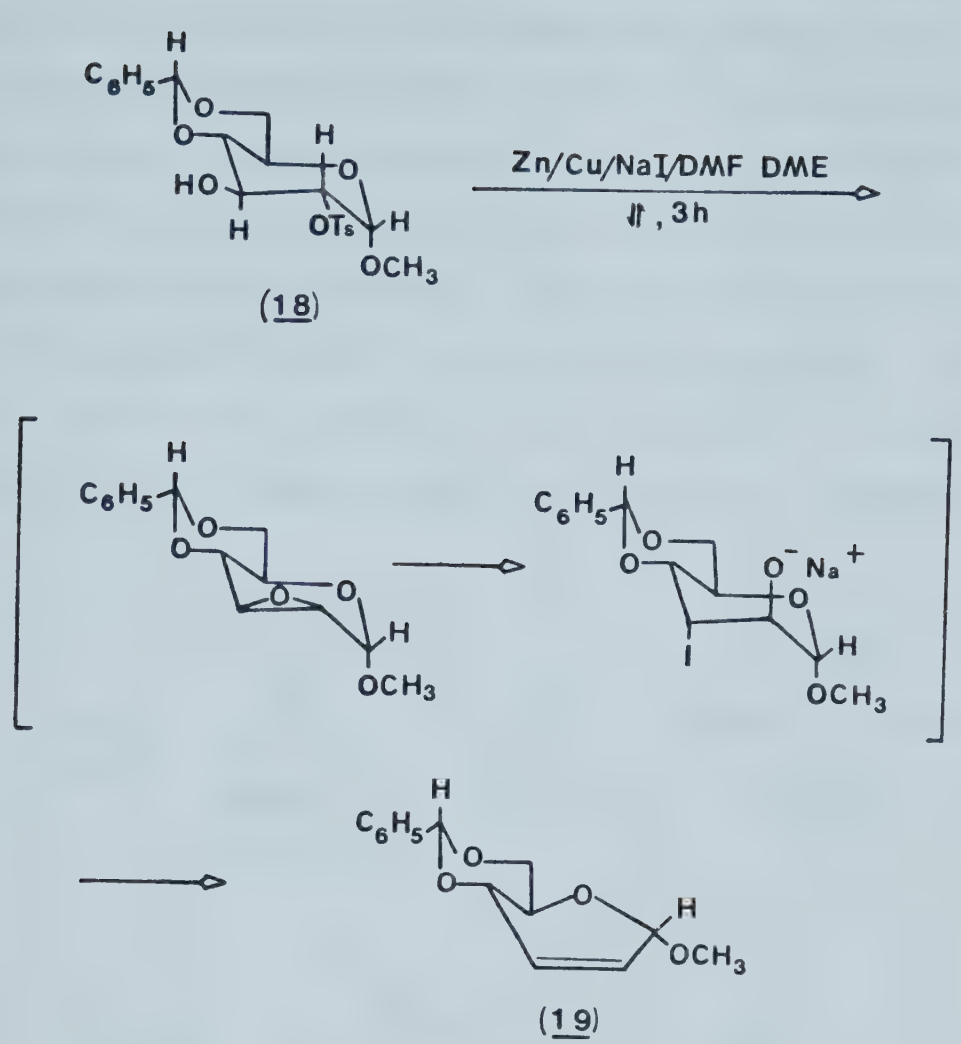
In 1980, Radatus and Clarke[78] noted that treatment of methyl 4,6-O-benzylidene-2-O-tosyl-α-D-glucopyranoside[79] (18) with a mixture of zinc-copper couple, sodium iodide, dimethylformamide and dimethoxyethane under reflux gave methyl 4,6-O-benzylidene-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside(19). (scheme J) The basic reaction conditions were those used in the Tipson-Cohen reaction.[58] Addition of dimethoxyethane maintained the reaction temperature at 125-130°C and caused precipitation of the Lewis acid zinc(II) iodide which induces decomposition of the olefin.[80] Reaction proceeds via the manno epoxide[78] which is cleaved



SCHEME I

with sodium iodide to give the iodo-alkoxide. This undergoes a Boord reaction[81-83] in the presence of zinc.

In 1981, Panzica *et al.*[84] reported a vicinal diol to olefin synthesis based on the Garegg and Samuelsson[77] reaction. In that procedure, triiodoimidazole was used but its preparation is quite cumbersome on a large scale.[77] The modified route avoids this drawback by treatment of the diol with triphenylphosphine, iodoform and imidazole. This method



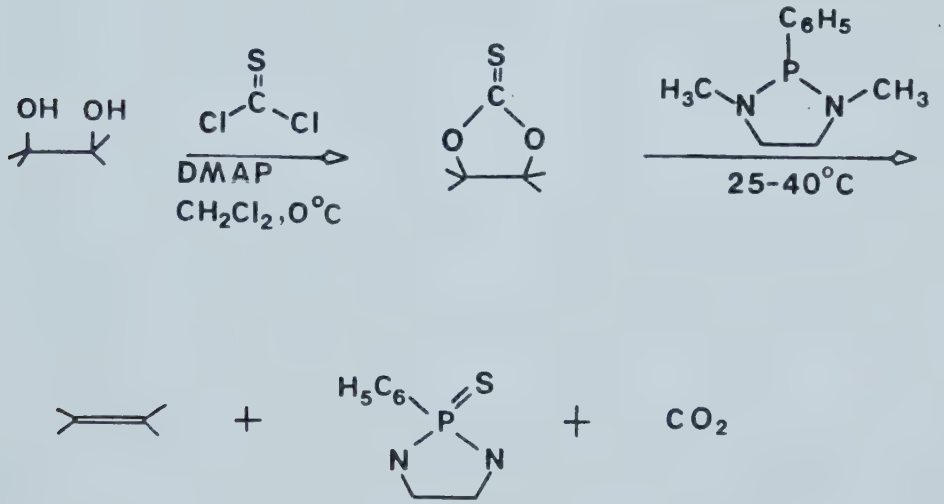
(18)

(19)

SCHEME J

was found to be applicable to sugars containing cis- or trans-1,2-diols. The authors proposed a mechanism involving iodide displacement of the oxophosphonium group resulting in the formation of a vicinal di-iodo derivative. Reductive elimination of the di-iodo intermediate with imidazole furnished the unsaturated product. No unsaturated product was observed in the absence of imidazole. Replacement of imidazole by zinc gave olefins in poor yield.

In 1982, Corey *et al.*[45] reported an improved procedure for the stereospecific synthesis of olefins from 1,2-diols via cyclic thionocarbonate esters.[47] Treatment of a solution of diol and 4-dimethylaminopyridine in methylene chloride at 0°C with thiophosgene gave the thionocarbonate. This was converted to the alkene at 25-40°C by treatment with 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidene[46] (rather than the earlier procedure[47] that employed trimethylphosphite or triethylphosphite at reflux). (scheme K)



SCHEME K

The authors used this procedure in a synthesis of erythronolide A and recommended its use in cases involving complex or sensitive molecules.

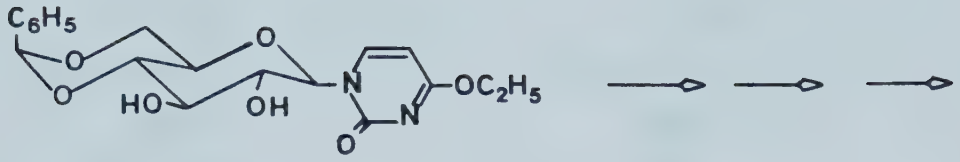
Barva and Sharma[87] described a one-step method for transforming cis or trans secondary and tertiary vicinal

diols to olefins. This method involves treatment of a solution of diol and sodium iodide in dry acetonitrile with chlorotrimethylsilane. The reaction is carried out at room temperature. The authors indicate that this method is superior to other literature methods since alkenes can be obtained in high yields at low temperature under mild neutral conditions using inexpensive reagents.

C. Preparation of Unsaturated Nucleosides

A number of routes have been described for the preparation of 2',3'-unsaturated nucleosides. One of the earliest publications by Stevens *et al.*[88] described the synthesis of 1-(4,6-O-benzylidene-2,3-dideoxy-β-D-erythro-hex-2-enopyranosyl)-4-O-ethyluracil (21) from 1-(4,6-O-benzylidene-β-D-glucopyranosyl)-4-O-ethyluracil (20). Compound (20) was converted to a 2',3'-epoxide (22) by 2'-O-monotosylation followed by acetylation and treatment with sodium ethoxide. The epoxide was opened with sodium iodide, acetic acid and sodium acetate to give the iodohydrin. This was mesylated (23) and treated with excess sodium iodide in acetone to give the first noted unsaturated nucleoside. (scheme L)

This general approach was used by Horwitz *et al.*[89] to prepare 2',3'-unsaturated uridine. The xylo epoxide (27) was formed by treatment of 2',3',5'-tri-O-mesyluridine (24) with sodium hydroxide[90] presumably via the two intermediates (25) and (26). (scheme M) Treatment of isolated (25)[91] or (26)[92] with aqueous sodium hydroxide gave (27). Opening of the epoxide ring of (27) with sodium iodide in acetone gave a single iodohydrin (28). This was converted to the mesylate (29) and subjected to elimination with iodide in acetone to yield (30). (scheme M)



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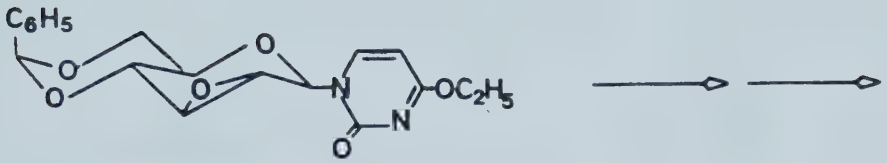

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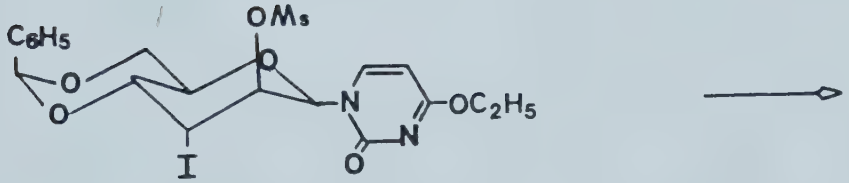

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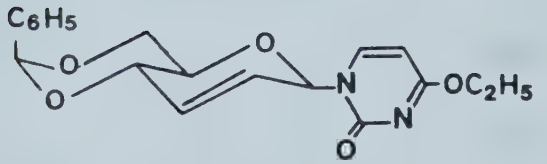
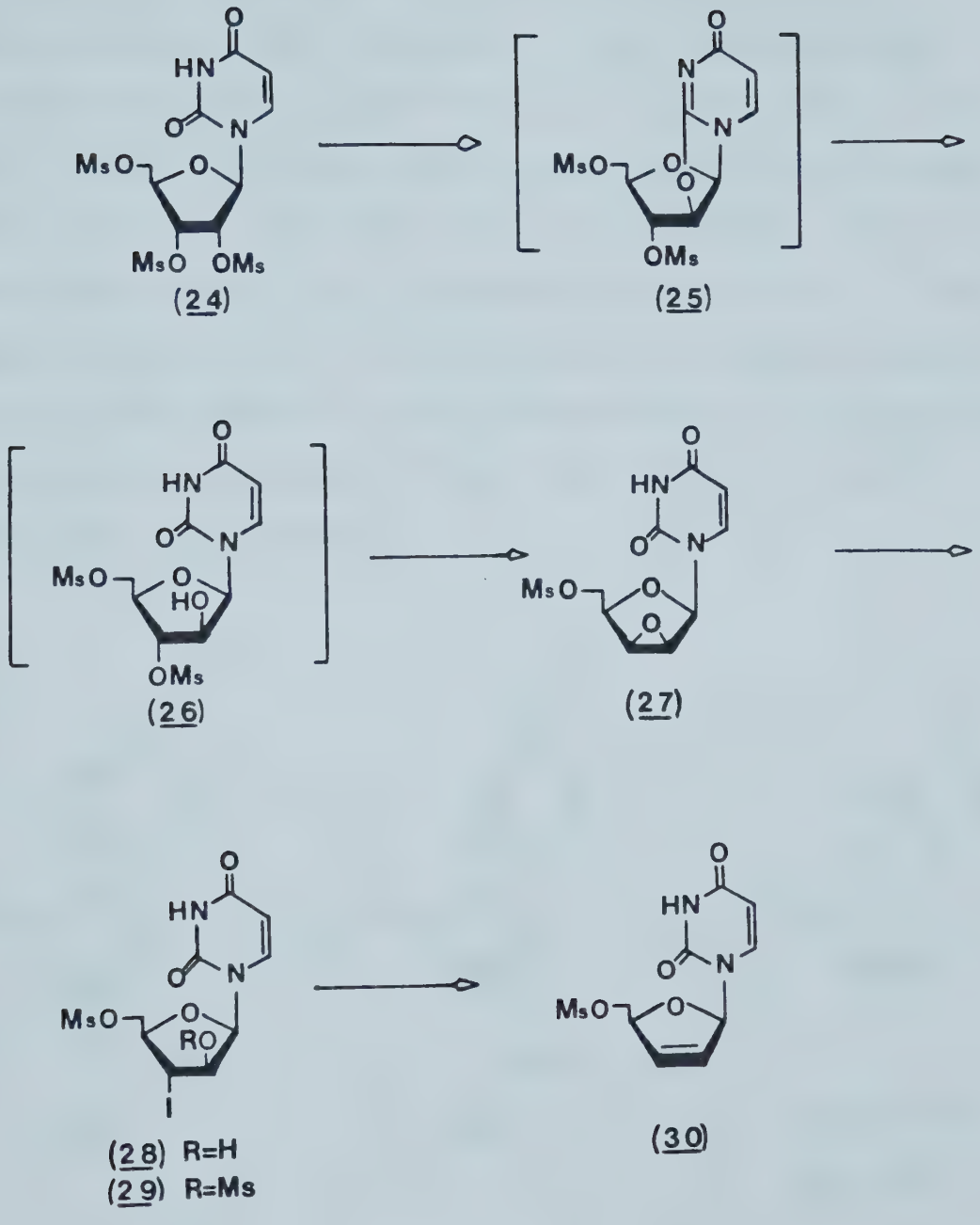


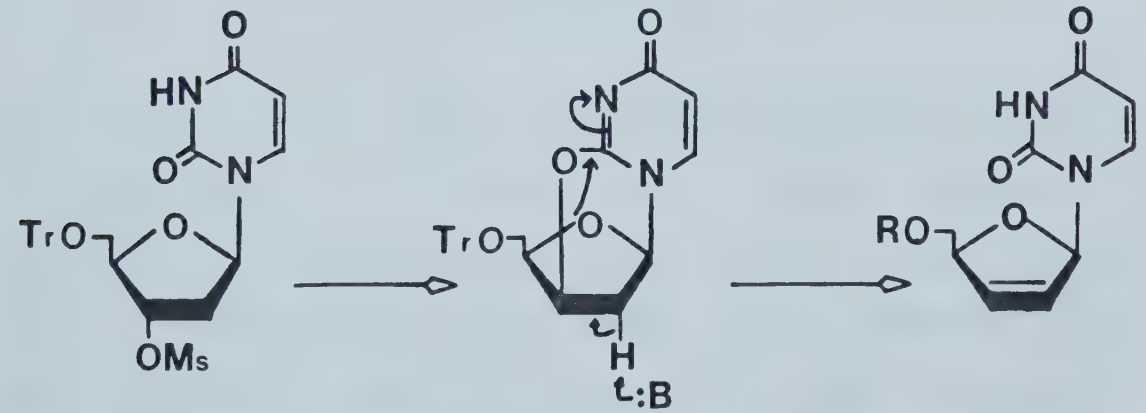
(21)

SCHEME L



SCHEME M

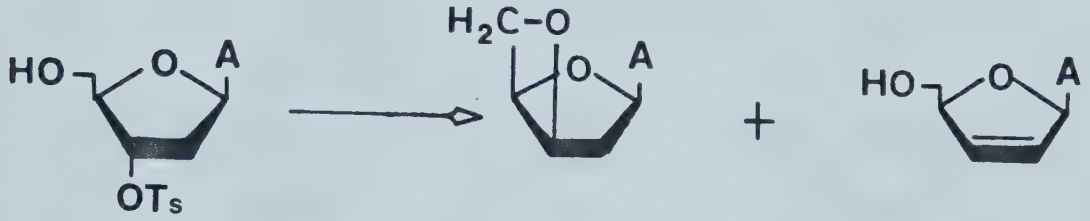
Horwitz *et al.*[33] treated 3'-O-mesyl-5'-O-trityl-2'-deoxyuridine (31) with sodium hydroxide in ethanol to give 2,3'-anhydro-1-(2-deoxy-5-O-trityl-β-D-threo-pentofuranosyl)uracil (32). Reaction of (32) with potassium t-butoxide in dimethyl sulfoxide at room temperature gave 1-(5-O-trityl-2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)-uracil (33) which was deprotected with hydrogen chloride in chloroform to give (34). (scheme N) Horwitz *et al.*[33] later demonstrated that this base-catalysed elimination reaction also could be applied to the 3'-mesylates of 1-(2-deoxy-β-D-threo-pentofuranosyl)pyrimidines (pyrimidines = thymine, uracil and 4-thiouracil).



SCHEME N

Similar base-catalysed elimination reactions have been used by others to obtain 2',3'-unsaturated nucleosides.

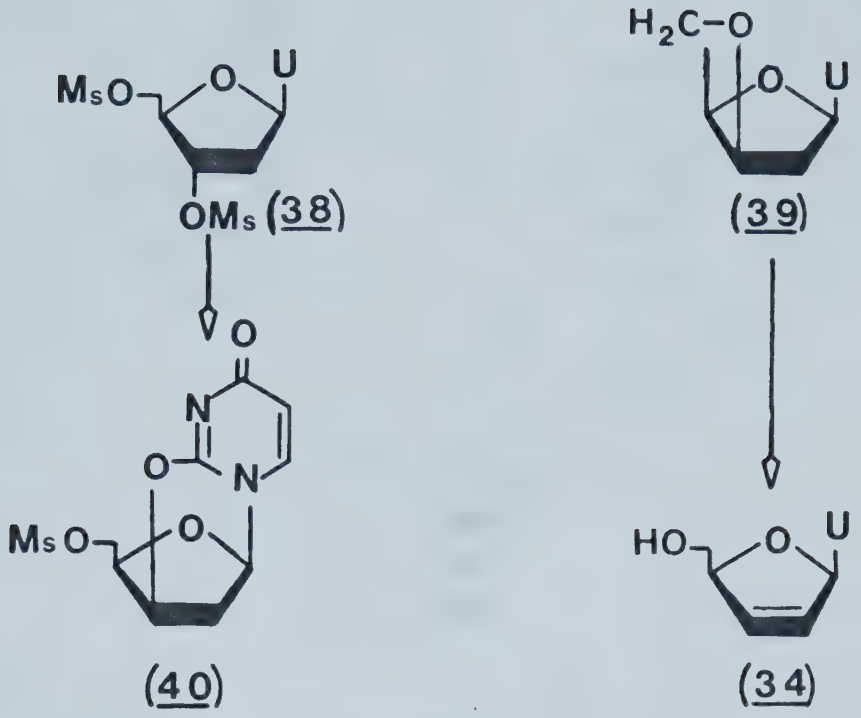
Robins *et al.*[44] treated 3'-O-p-toluenesulfonyl-2'-deoxyadenosine[45] (35) with sodium methoxide in DMF at room temperature to give a 2',3'-unsaturated adenosine product. Horwitz *et al.*[46] found that treatment of 3'-O-p-toluenesulfonyl-2'-deoxyadenosine (35) with sodium ethoxide in ethanol gave two products (36) and (37). (scheme O)



(35) (36) (37)

SCHEME O

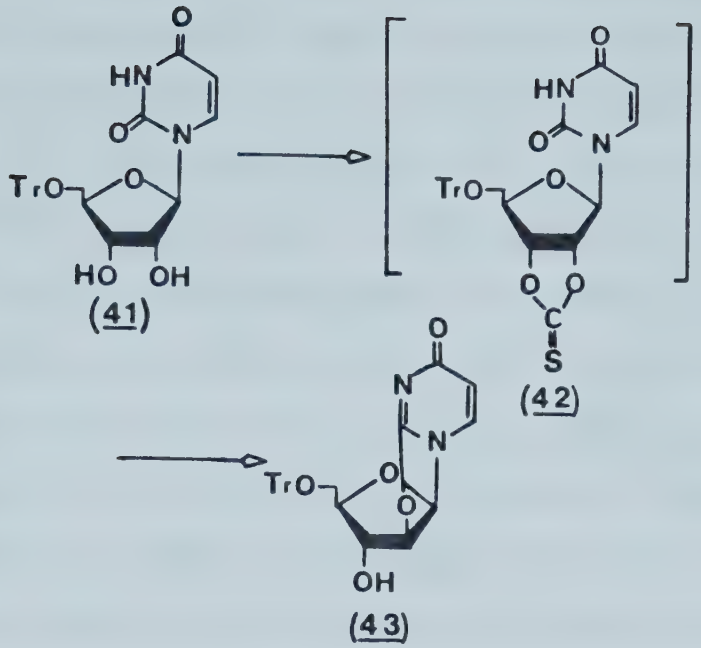
Inoue *et al.*[47] improved the base-catalysed conditions for formation of 2',3'-unsaturated uridine and thymidine compounds. They found that 3',5'-di-O-mesyl-2'-deoxyuridine (38) was converted into the oxetane (39) by treatment with sodium hydroxide at pH 12. The pH of the reaction mixture was found to be critical since at pH 9.0 the cyclonucleoside (40) was formed. Treatment of (39) with potassium t-butoxide in dimethyl sulfoxide gave the 2',3'-unsaturated product (34) in a yield of 40-60%. However, sodium hydroxide in hexamethylphosphoric triamide converted (39) into (34) almost quantitatively. (scheme P)



SCHEME P

In 1965, Fox and Wempen[18] attempted application of the Corey-Winter[17] method to uridine. Treatment of 5'-O-trityl-uridine (41) with thiocarbonyldiimidiazole in hot toluene gave 2,2'-anhydro-1-(5'-O-trityl-β-D-arabinofuranosyl)uracil (43). The authors proposed the 2',3'-thionocarbonate ester (42) as an intermediate in the conversion of (41) to (43). It was suggested that the thionocarbonate (or 2'-O-thiocarbonylimidazolide intermediate) can behave as a leaving group for attack of the 2-carbonyl at C'2 under these reaction conditions. (scheme Q)

Patchett *et al.*[19] also attempted to prepare 2',3'-unsaturated nucleosides by application of the two-stage olefin synthesis of Corey and Winter[17]. Their results paralleled those of Fox and Wempen,[18] but in



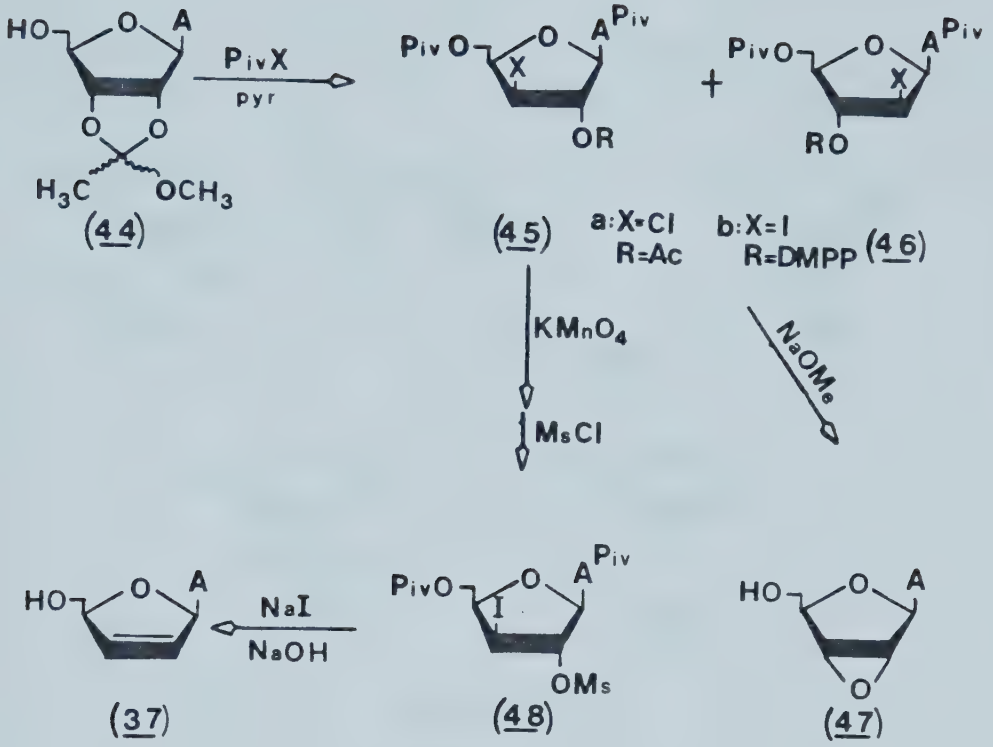
SCHEME Q

addition, compound (42) (scheme Q) was isolated by conducting the reaction of (41) with thiocarbonyldiimidazole in tetrahydrofuran at room temperature. Treatment of (42) with imidazole (in refluxing toluene) or potassium t-butoxide in ethanol gave (43). Conversion of (42) to the 2',3'-unsaturated-5'-O-trityluridine derivative was effected by heating with inactivated Raney nickel. A low yield (no value reported) was obtained and a considerable amount of the cyclonucleoside (43) was produced in the reaction.

Robins *et al.*[100] found that treatment of 2',3'-O-methoxyethylideneadenosine (44) with pivalic acid chloride in refluxing pyridine gave a mixture containing 6-N-pivalamido-9-(3-chloro-3-deoxy-2-O-acetyl-5-O-pivalyl-β-D-xylofuranosyl)purine (45a) and its

2'-chloroarabino isomer (46a) as the major products. (scheme R) Treatment of (45a) or (46a) with methanolic sodium methoxide gave 9-(2,3-anhydro-β-D-ribofuranosyl)-adenine (47). Treatment of (45a) and (46a) with tri-n-butyltin hydride and azobisisobutyronitrile gave 2'-deoxy-adenosine and 3'-deoxyadenosine. Treatment of (44) with sodium iodide and pivalyl chloride in pyridine gave 6-N-pivalamido-9-(3-iodo-3-deoxy-2-0-[4,4-dimethyl-3-pivaloxy-pent-2-enoyl]-5-0-pivalyl-β-D-xylofuranosyl)purine (45b) and its 2'-iodo-3'-0-DMPP isomer (46b). The DMPP group was selectively removed from (45b) using potassium permanganate in aqueous pyridine at 0°C. The resulting 6-N-pivalamido-9-(3-iodo-3-deoxy-5-0-pivalyl-β-D-xylofuranosyl)purine was converted to the 2'-mesylate (48). Treatment of crude (48) with cold aqueous base containing sodium iodide resulted in elimination of iodine and mesylate with concomitant deprotection to give 9-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)adenine (37)[101]. (scheme R)

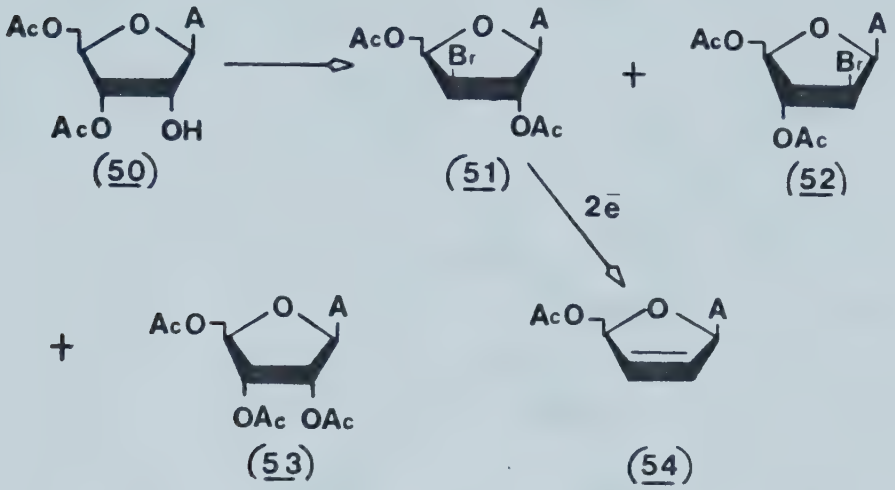
Inoue *et al.*[102] treated 3',5'-di-0-acetyladenosine (50) with tetraacetyoxysilane and phosphorus tribromide in the presence of boron trifluoride etherate and obtained 9-(2,5-di-0-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)adenine (51) in 47% yield. (scheme S) A small amount of the 2'-bromo isomer (52) and 2',3',5'-tri-0-acetyladenosine (53) also were formed. The synthesis of 2',3'-unsaturated adenosine, cytidine and uridine compounds was accomplished by electrochemical reduction of the vicinal bromo esters.



SCHEME R

Cathodic reduction of (51) (scheme S) was effected at -1.45V vs SCE in dimethylformamide (DMF) with tetrabutylammonium bromide (TBA.Br) as supporting electrolyte. The desired 9-(5-O-acetyl-2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)-adenine (54) was obtained in 77% yield. Electrolysis of 3',5'-di-O-propionyl-2'-bromo-2'-deoxyuridine (55) in DMF with tetraethylammonium tosylate as electrolyte afforded 1-(5-O-propionyl-2,3-dideoxy-β-D-glycero-pent-2-eno-

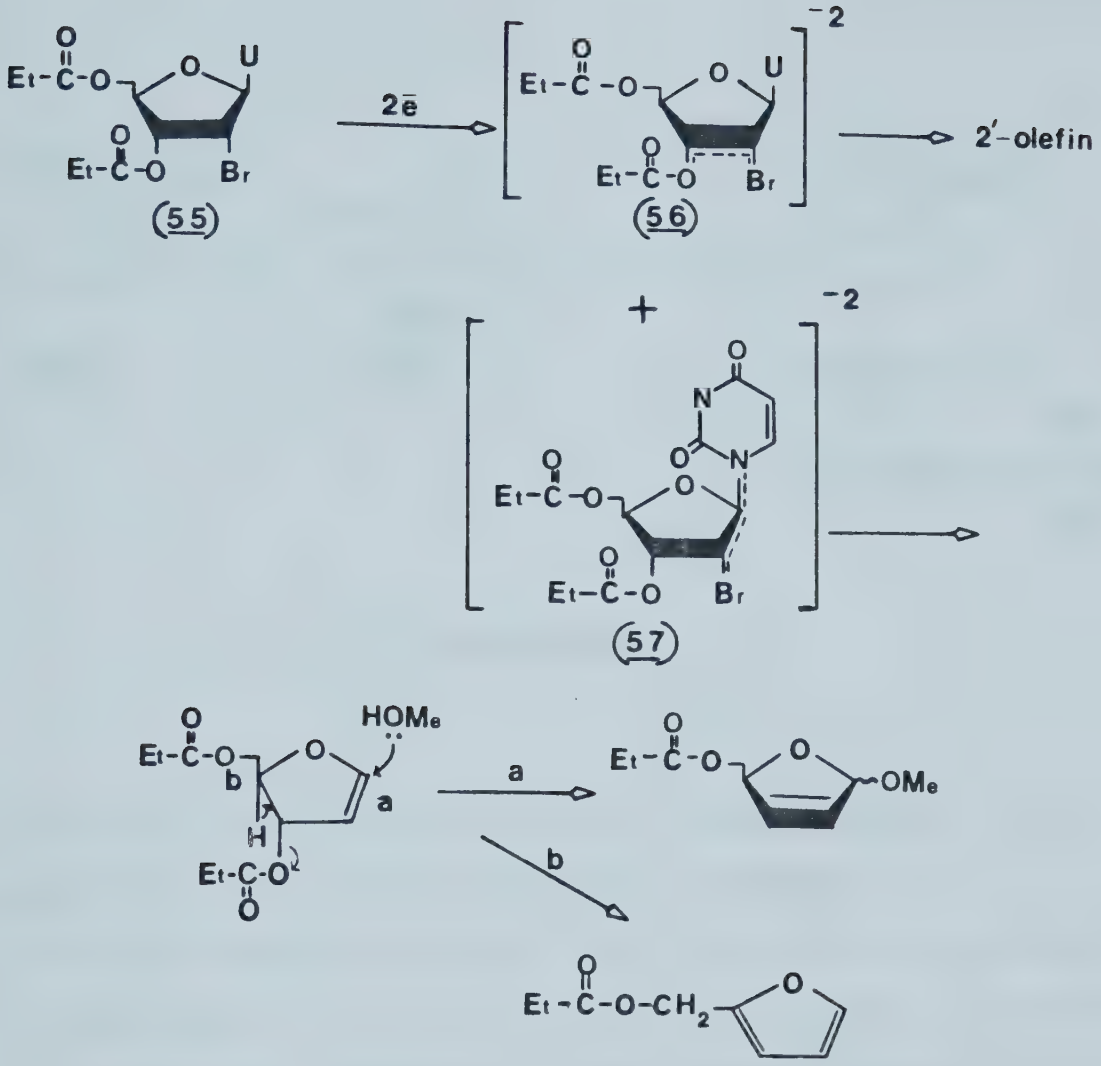
furanosyl)uracil in 70% yield. However, appreciable glycosidic cleavage leading to uracil was observed in this case.[103]



SCHEME S

The authors suggested a concerted two electron reduction mechanism for the formation of both the 2'-olefin and glycosidic cleavage. Two energetically close transitions (56) and (57) would produce the 2'-olefin and glycosidic cleavage. (scheme T)

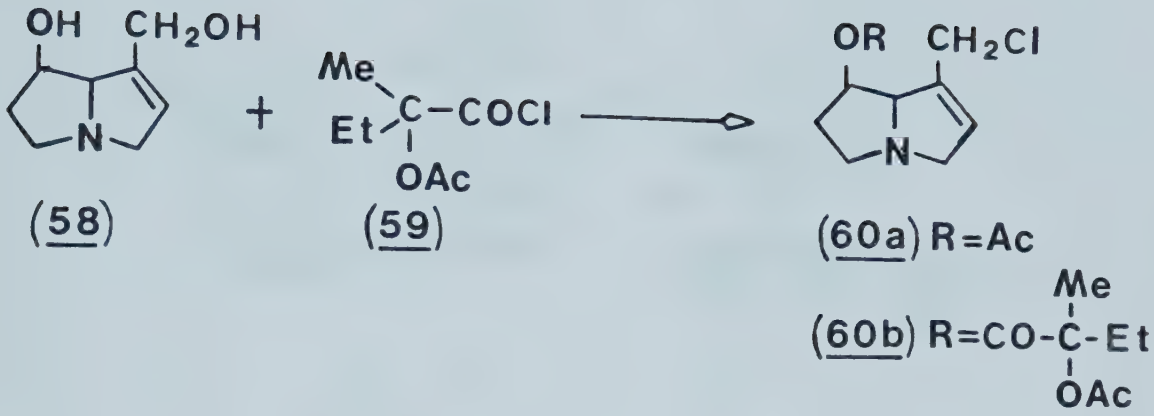
Mattocks'[104] observed an "abnormal reaction" involving the 1,4-diol (58) and 2-acetoxy-2-methylbutanoyl chloride (59). The unexpected products of this reaction were the chloroesters (60a) and (60b). It was suggested that nucleophilic attack upon α-acyloxy acid chlorides with bulky substituents at the α position occurred primarily at the acetoxy carbonyl group rather than the acyl chloride



SCHEME T

carbonyl. (scheme U)

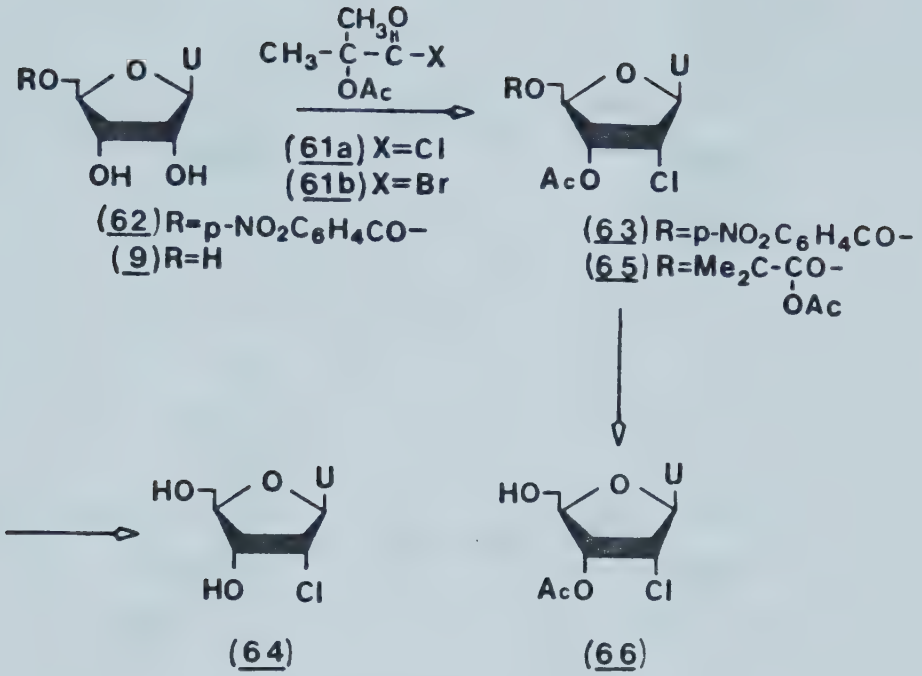
Moffatt *et al.*[105] applied these observations of Mattocks[104] for selective transformations of the vicinal diol grouping in ribonucleosides. Since 2-acetoxy-2-methyl-butanoyl chloride has a chiral centre, they substituted the closely related α-acetoxyisobutyryl chloride (61a) in their work. They treated cis-cyclopentane-1,2-diol with 1.2-1.5 equivalents of (61a) in solvents such as ether or



SCHEME U

acetonitrile and obtained a single product identified as trans-2-chlorocyclopentyl acetate. Treatment of 5'-O-(p-nitrobenzoyl)uridine (62) with 4 molar equivalents of (61a) at 100°C for 45 min gave the halogenated nucleoside (63). The ^{1}H nmr spectrum of this product indicated that the 3'-hydroxyl group was acylated since the C3'-H appeared at lower field than that of C2'-H. Deacylation of this material gave the known 2'-chloro-2'-deoxyuridine[10] (64). (scheme V)

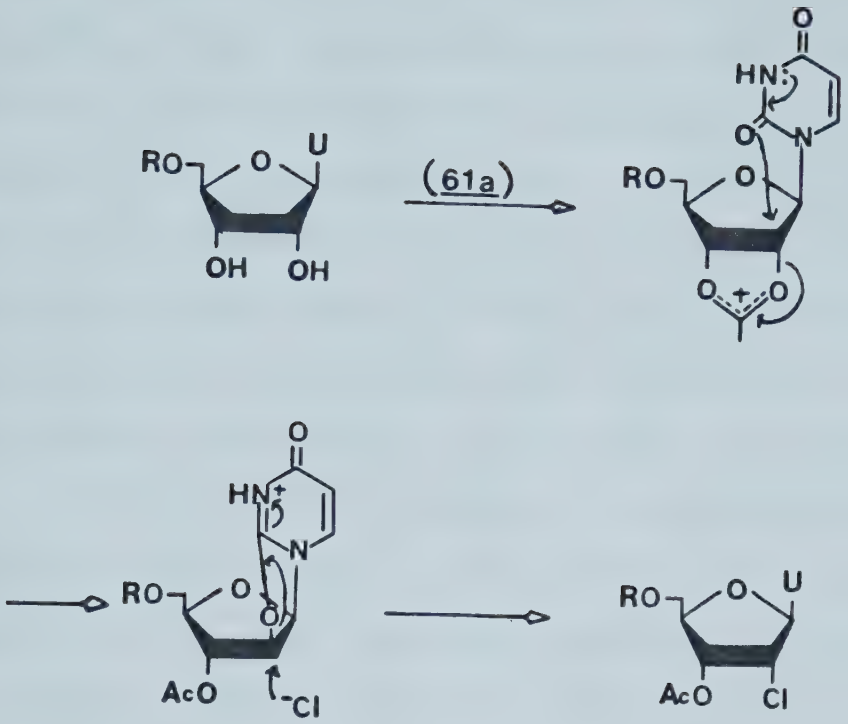
Treatment of unprotected uridine (9) with (61a) in acetonitrile at 80°C gave a mixture from which the major product (65) was isolated in 74% yield. Treatment of the crude reaction product with methanolic hydrogen chloride at room temperature followed by crystallization, gave



SCHEME V

3'-O-acetyl-2'-chloro-2'-deoxyuridine (66) in an overall yield of 59% from uridine. The overall cis stereochemistry is a consequence of double inversion involving participation of the C2 carbonyl group of the uracil ring. (scheme W)

Treatment of adenosine (6) with 3-4 molar equivalents of (61a) in acetonitrile at 80°C for one hour resulted in approximately 20% glycosyl cleavage to adenine under the acidic conditions. Similar results were obtained with

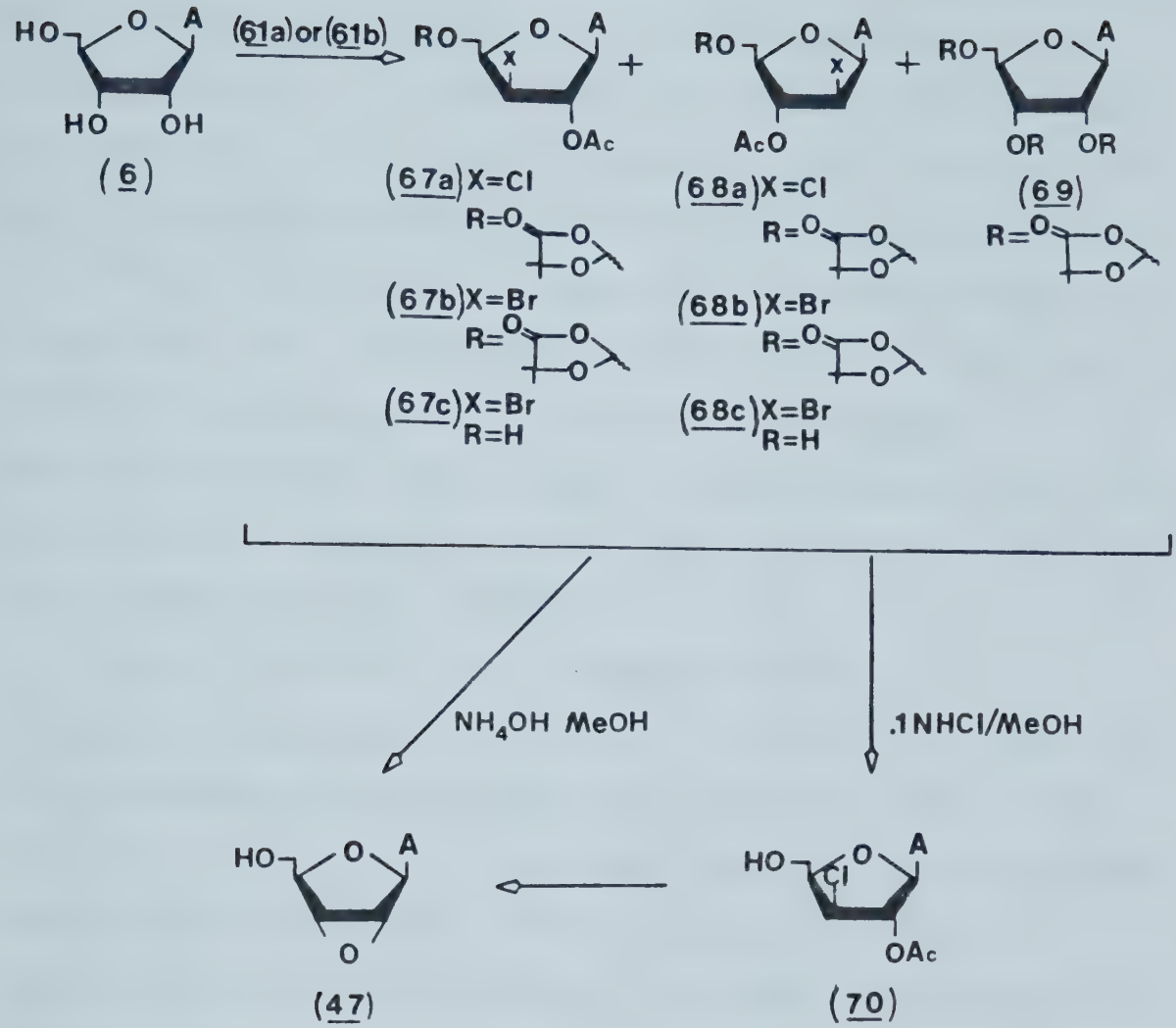


SCHEME W

reactions carried out at lower temperatures for substantially longer periods of time. The major product was 9-[2-O-acetyl-3-chloro-3-deoxy-5-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)-β-D-xylofuranosyl]adenine (67a) and the minor product was 9-[3-O-acetyl-2-chloro-2-deoxy-5-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)-β-D-arabinofuranosyl]adenine (68a). Treatment of the crude reaction mixture with 0.1 N methanolic hydrogen

chloride resulted in removal of the O5' substituent. Crystallization gave 9-(2-O-acetyl-3-chloro-3-deoxy-β-D-xylofuranosyl)adenine (70) in 60% yield. This compound was assigned the xylo configuration by its facile conversion into 9-(2,3-anhydro-β-D-ribofuranosyl)adenine (47) upon treatment with sodium methoxide. (scheme X)[106] Treatment of the mother liquors with 10% methanolic ammonium hydroxide following crystallization of (67a) led to formation of (47) along with some adenosine (6) and deprotected chloro nucleosides. The precursor of this adenosine presumably was 2',3',5'-tris-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl) adenosine (69) formed as a by-product in the initial reaction of (6) with α-acetoxyisobutyryl chloride (61a).

Since catalytic hydrogenolysis of the chloro sugars was unsuccessful, Moffatt and coworkers[106] investigated formation of the corresponding bromo derivatives. Treatment of the acyl chloride (61a) with anhydrous lithium bromide in ethyl acetate gave the acyl bromide (61b) in 63% yield. Reactions of this acyl bromide (61b) with adenosine (6) were more rapid. Adenosine underwent complete reaction in approximately 30 min at room temperature in acetonitrile and only 2-3% adenine cleavage occurred. Examination of this reaction mixture by tlc showed two major spots in an approximate ratio of 5:1. The minor product was identified as 2',3',5'-tris-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)-adenosine (69). The major fraction consisted of a mixture of the 3-bromo-xylo (67b) and 2-bromo-arabino (68b) compounds.



SCHEME X

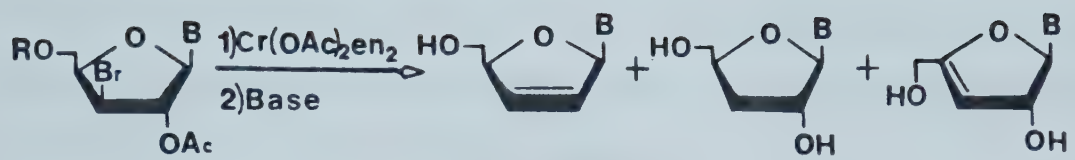
(scheme X) Moffatt *et al.*[107] have extended this work to tubercidin (71) and formycin. They[108] also reported treatment of inosine (72) and guanosine (7) with α-acetoxy-isobutyryl bromide to obtain the 2'- and 3'-trans halo-acetates. Experimental details of this work have not been published.

Moffatt *et al.*[107] developed a direct 2',3'-unsaturation procedure from these vicinal halo acetates via an

elimination reaction using chromous acetate. The preparation of chromous acetate begins with chromous perchlorate and sodium acetate. It is unstable in air and must be prepared and used in a dry box under nitrogen or argon. Treatment of the 5'-blocked vicinal halo acetate with 5 molar equivalents of chromous acetate and 10 equivalents of ethylenediamine in ethanol at -78°C for 30 min followed by deprotection with methanolic ammonia gave 9-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)adenine (37), 3'-deoxyadenosine (73) and 9-(3-deoxy-β-D-glycero-pent-3-enofuranosyl)adenine (74) in the yields indicated. (scheme Y)

Results observed with analagous treatment of the vicinal halo acetates from inosine, tubercidin, uridine and O5',N^2-dibenzoylguanosine also are given in scheme Y. Thus chromous ion reduction of the halo function competes to some degree with the desired reductive elimination reaction. Similar results were found using chloro and iodo acetates in place of the bromo acetates.

Recently Garegg and Samuelsson[110] reported treatment of the adenosine bromo acetate derivative described by Moffatt and coworkers[109] with zinc powder in ethanol containing acetic acid. Deacylation afforded the 2',3'-unsaturated adenosine in 69% yield. Application of this method to 5'-O-(2-acetoxyisobutyryl)-3'-O-acetyl-2'-bromo-2'-deoxyuridine gave the 2',3'-unsaturated uridine in 52% yield.



B=adenine R=trimethyldioxolanone	59%	30%	10%
B=hypoxanthine R=trimethyldioxolanone	53%	3%	
B=N_2-Bz-guanine R=Bz	24%		
B=tubercidin R=trimethyldioxolanone	43%	9%	
B=uracil R=Me_2-C(OAc)-CO	33%	3%	

SCHEME Y

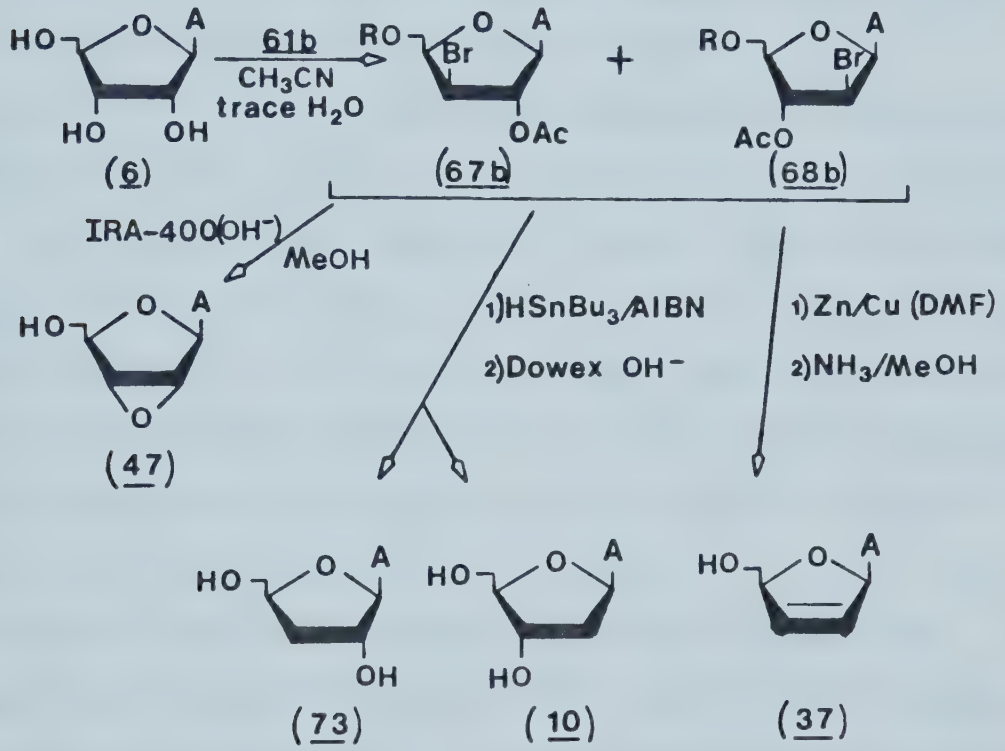
D. Results and Discussion

The goal of this thesis research was to develop a simple, efficient route to 2',3'-unsaturated nucleosides. Our first step involved improvement of the preparation of α-acetoxyisobutyryl bromide (61b)[100]

Since α-acetoxyisobutyryl bromide is very sensitive to moisture, the glassware used in its preparation was oven dried, assembled hot and allowed to cool while purged with a stream of dry nitrogen. Solvent ethyl acetate was distilled twice from P_2O_5 and the lithium bromide was vacuum dried at 110°C for 72 h. Employing these conditions, a clear solution of lithium bromide in ethyl acetate was obtained. Addition of α-acetoxyisobutyryl chloride (61a) was followed by distillation to give consistent yields of 90-92% of (61b).

Moffatt *et al.*[100] had reported that approximately 20% of the product obtained upon treatment of adenosine (6) with (61b) was 2',3',5'-tris-O-(2,5,5-trimethyl-1,3-dioxolan-4-on-2-yl)adenosine (69). We found that addition of a small amount of water to the reaction mixture eliminated formation of this by-product. Our conditions consisted of treating 1 mmol of dried adenosine (6) in 20 mL of dry acetonitrile with 2 mL acetonitrile/water (100:1) and then 4 mmol of α-acetoxyisobutyryl bromide (61b). Upon work-up, ultraviolet spectral analysis of the aqueous layer indicated approximately 2% adenine. Thin layer chromatography of the

organic phase showed two major products. Treatment of this mixture with IRA 400(OH^-) resin in absolute methanol gave one product cleanly (92% yield overall from adenosine) which was identified as 9-(2,3-anhydro-β-D-ribofuranosyl)-adenine[100] (47). (scheme Z) This reaction sequence represents a significant improvement over the previously preferred method for the preparation of this compound.[100]



SCHEME Z

Treatment of the above noted organic phase mixture with tri-n-butyltin hydride and AIBN followed by deacylation gave 3'-deoxyadenosine (73) and 2'-deoxyadenosine (10) in 80% and 10% yields, respectively. (scheme Z) These conversions were in harmony with spectral data that identified the two major organic soluble products as 9-(2-O-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)- and 9-(3-O-acetyl-2-bromo-2-deoxy-β-D-

arabinofuranosyl)adenine and their 5'-O-(2,5,5-trimethyl-dioxolan-4-on-2-yl) derivatives (67b, 68b).[106] The above sequence also provides a simple and high yield synthesis of the antibiotic cordycepin (3'-deoxyadenosine).

The use of zinc to effect reductive elimination of β-ethoxyalkyl bromides (β-bromo ethers) to give olefins was pioneered by Boord *et al.*[81] Other researchers have used zinc-copper couple for analagous preparations of olefins.[82,83,111-114] The stereochemistry of elimination reactions using metals with halohydrins or their derivatives has been studied. The reaction proceeds readily with both trans- and cis-2-bromocyclohexanol.[112] Two mechanisms have been proposed[115] for the β-halo ether reductive elimination. The first involves formation of a free-radical intermediate. This mechanism is consistent with the lack of stereo-specificity observed in acyclic systems. However, these elimination reactions appear to proceed without the coupling, disproportionation and solvent attack expected for radical intermediates.[116] A further serious objection to the radical process is the question of whether a radical would spontaneously eliminate an alkoxy radical. The reverse reaction appears to occur readily in the peroxide-catalysed addition of hydrogen bromide to olefins.[117] Consequently, the non-stereospecific elimination reaction probably proceeds via organometallic or carbanionic intermediates. Both the failure to detect radical intermediates[118,119] and the accompanying reduction of the carbon-bromide bond when

the reaction is carried out in hydroxylic solvents supports a carbanion-type mechanism.[115]

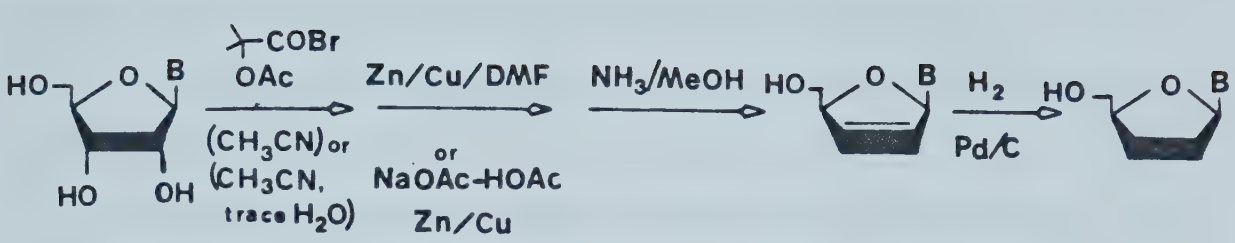
Conversion of the bromo acetate mixture (67b, 68b) to the corresponding 2',3'-unsaturated product was first attempted using zinc-copper couple in a sodium acetate/acetic acid buffer solution at -10 to -20°C. Although this method resulted in formation of the desired product (the 2',3'-unsaturated adenosine in 80% yield), the work-up procedure was laborious (as illustrated by the procedure with uridine given in the Experimental section). It was also found that considerable care had to be taken with temperature control to avoid glycosidic cleavage versus freezing of the mixture. In order to circumvent these problems, various solvents were examined. Use of protic solvents such as ethanol and methanol resulted in formation of significant amounts of 3'-deoxyadenosine (10% with ethanol and 12% with methanol). When DMF was used as solvent, the reductive elimination proceeded smoothly.

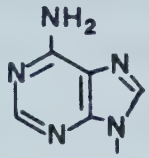
The overall sequence involves treatment of adenosine (6) with α-acetoxyisobutyrl bromide (61b) in moist acetonitrile and partitioning of the product between ethyl acetate and aqueous sodium bicarbonate solution. Treatment of the organic phase with acetic anhydride and 4-(dimethylamino)pyridine (DMAP) protects any free 5'-hydroxyl groups as 5'-acetates. A DMF solution of this mixture is treated with freshly prepared (and DMF washed) zinc-copper couple at room temperature. The zinc-copper

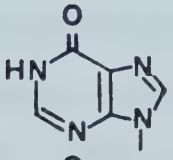
couple is removed by fitration using celite. Evaporation of the DMF and crystallization of the residue gave (37) in 81% yield overall from (6).(scheme AA)

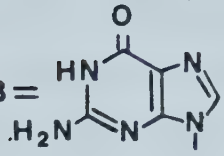
We then extended this reaction sequence (beginning with α-acetoxyisobutyryl bromide) to other ribonucleosides. The procedures for tubercidin (71), toyocamycin (75) and sangivamycin (76) (scheme AA) followed that for adenosine. Overall yields of the 2',3'-unsaturated nucleosides 4-amino-7-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)-pyrrolo[2,3-d]pyrimidine (77) and its 5-cyano (78) and 5-carboxamido (79) analogues were 90%, 81% and 80%, respectively. The 2',3'-unsaturated analogues of toyocamycin (78) and sangivamycin (79) were hydrogenated to give the previously unknown 2',3'-dideoxy compounds (80) and (81) in high yields.

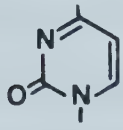
The two remaining purine nucleosides, inosine (72) and guanosine (7), gave very poor yields when exposed to the reaction conditions used for adenosine. Treatment of inosine with 8 equivalents of (61b) under anhydrous conditions at room temperature for 3 h gave good results. Ultraviolet analysis of the aqueous extract showed approximately 4% cleavage to hypoxanthine. The crude organic phase was treated with IRA-400(OH^-) resin in absolute methanol followed by elution and chromatography on silica gel to give the inosine epoxide in 80% yield. This again shows a marked improvement over previously published methods for preparation of this compound.[120]

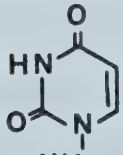


(6) B =  (37)

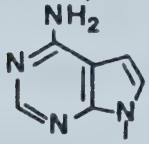
(72) B =  (82)

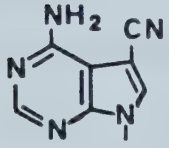
(7) B =  (83)

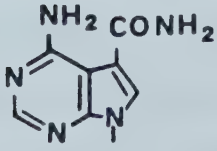
(84) B =  (85)

(9) B = 

(34)

(71) B =  (77)

(75) B =  (78) (80)

(76) B =  (79) (81)

SCHEME AA

Treatment of the processed organic phase with zinc-copper couple followed by deprotection using Dowex(OH^-) resin, elution and silica gel chromatography gave 9-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)hypoxanthine (82) in 70% yield overall from inosine.

The same procedure that was used for inosine was followed in the guanosine series. Guanosine undergoes glycosidic cleavage most readily of all the purine-type nucleosides that we investigated.[12] Lack of solubility in acetonitrile also was a major problem in the guanosine case.

Treatment of guanosine with α-acetoxyisobutyryl bromide in acetonitrile under anhydrous conditions for 3 h at room temperature gave the best ratio of guanosine bromoacetate derivatives to guanosine plus guanine (resulting from glycosidic cleavage). Ultraviolet spectral analysis of the aqueous layer indicated approximately 30% of the unreacted guanosine plus guanine. Treatment of the organic soluble products with zinc-copper couple followed by an identical work-up used for inosine gave 9-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)guanine (83) in 47% yield overall from guanosine. Owing to the instability of this compound, satisfactory elemental analytical data were not obtained.

Both of the pyrimidine nucleosides, uridine and cytidine, were converted to their 2',3'-unsaturated derivatives. Moffatt's[10] procedure for the preparation of 5'-O-(2-acetoxyisobutyryl)-3'-O-acetyl-2'-bromo-2'-deoxy-

uridine and 3',5'-di-O-acetyl-2'-bromo-2'-deoxyuridine was followed using 4 equivalents of α-acetoxyisobutyryl bromide in acetonitrile at 80°C for 3 h. The above mentioned two products were isolated in virtually quantitative yield. Treatment of this mixture with zinc-copper couple in sodium acetate/acetic acid at -15°C followed by neutralisation with sodium bicarbonate and an identical work-up to the one used for inosine gave 1-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)uracil (34) in 50% yield. The lower yield in this case is presumably due to glycosidic cleavage in the acetic acid during work-up. However, it could also be due to the competing glycosidic cleavage during reduction with the zinc-copper couple.

Treatment of cytidine with α-acetoxyisobutyryl bromide under the conditions developed for adenosine did not proceed to give the cytidine bromoacetates. Treatment for a longer period of time under the anhydrous conditions used for inosine also gave poor results. Selective acetylation of cytidine at N4'[22] and subjection of this derivative (84) to the conditions used for adenosine gave satisfactory results. Treatment of the presumed crude bromoacetate product with zinc-copper couple followed by the same work-up procedure that was used for adenosine gave a 70% yield of 1-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)cytosine (85).

The structures of the unsaturated nucleosides were examined by nmr spectroscopy. The 2' and 3' alkene hydrogens

had vinyl coupling constants of approximately 6Hz in each of these compounds. Table 1 contains the first order ^{1}H nmr coupling constants observed. ^{13}C nmr data for the unsaturated nucleosides is collected in Table 2.

An interesting finding resulted from subjection of 3'-deuterioadenosine[123] (86) to the overall sequence to give 9-(3-deuterio-2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)-adenine (87). The lower field vinyl proton signal was absent from the ^{1}H nmr spectrum of this compound and the corresponding lower field vinyl carbon signal intensity was depleted. Independent evaluation of a second purine nucleoside labelled with a 2'-deuterium corroborated assignment of the vinylic H2' and C2' to higher field in the 2',3'-unsaturated nucleosides.

We found that reduction of (87) resulted in reversal to the usual lower field positions for H2',2" and C2' relative to H3',3" and C3'. These labelling studies allowed correction of previous nmr spectral assignments that were based by analogy with the usual 2'<3' ordering.[84-97,103-109,124] From data presently available it appears that the general shift trend of H1'<H3'<H2'<H4'<H5',5" and C3'<C2'<C1'<C4'<C5' exists for the 2',3'-unsaturated nucleosides, although the close shifts for C1'(138.2) and C4'(138.0) of (87) might be reversed with other related compounds.

The presently developed sequence for the synthesis of 2'-enofuranosyl nucleosides from the parent ribonucleosides

provides a facile and relatively high yielding method for obtaining this class of compounds. It also gives convenient access to the biochemically important DNA chain terminator 2',3'-dideoxynucleosides by hydrogenation (or tritiation) of their 2',3'-unsaturated precursors.

TABLE 1

NMR Spin-Spin Coupling Values for 2',3'-Dideoxy-β-D-glycero-pent-2'-enofuranosylnucleosides[a]

Compound	$J_{1'-2'}$	$J_{2'-3'}$	$J_{3'-4'}$	$J_{4'-5'a}$	$J_{4'-5'b}$	$J_{1'-3'}$	$J_{1'-4'}$	$J_{2'-4'}$	$J_{5'-5'OH}$
37	1.5	6.0	2.1	3.5	3.7	1.5	2.9	1.5	5.3
77	1.7	5.8	2.0	3.6	3.6	1.7	2.5	2.0	5.5
85	1.5	6.1	1.8	3.8	3.9	1.4	3.1	1.8	-
82	1.3	5.7	2.2	3.3	4.0	1.7	3.0	1.4	5.1
83	1.8	6.5	2.1	4.1	4.2	1.4	3.3	1.6	5.5
78	1.6	5.8	1.9	3.9	3.8	1.5	2.3	2.1	5.7
79	1.2	5.6	2.3	3.6	3.8	1.6	2.8	1.9	5.4
34	1.5	6.1	2.0	3.4	3.4	1.5	3.1	1.5	-

[a]First order "apparent" coupling values

TABLE 2

^{13}C-NMR Data for 2',3'-Dideoxy-β-D-glycero-pent-2'-enofuranosylnucleosides and their 2',3'-Dideoxy-β-D-glycero-pentofuranosyl analogues

Compound	C-6	C-2	C-4	C-4a	C-7a	C-8	C-5	C-1'	C-2'	C-3'	C-4'	C-5'	others
37	155.97	152.51	149.10	-	-	139.02	118.73	87.93	125.44	134.26	87.80	62.74	
87	156.10	152.66	149.22	-	-	139.19	118.84	87.95	125.41	-	87.95	62.80	
82	156.54	145.85	147.90	-	-	138.38	123.95	88.19	125.15	134.58	87.94	62.59	
83	157.03	154.02	151.14	-	-	135.56	115.56	88.10	125.66	134.55	87.55	63.19	
77	121.10	151.71	157.46	102.64	149.84	-	99.70	87.57	126.15	133.63	87.08	63.50	
78	132.07	153.57	156.90	101.00	149.81	-	82.81	88.13	125.37	134.62	87.95	62.59	115.30
79	124.80	152.82	158.00	100.87	150.60	-	111.08	87.55	125.38	134.41	87.55	63.70	166.32
34	142.48	152.12	163.54	-	-	-	101.88	89.45	126.08	135.40	87.74	62.59	
85	141.46	155.31	165.60	-	-	-	93.98	89.72	126.67	133.89	87.00	62.62	
89	156.25	152.61	149.07	-	-	139.27	119.37	84.69	31.95	25.95	81.89	63.21	
90	155.97	152.35	148.77	-	-	139.02	119.07	84.43	31.64	-	81.58	62.90	
80	131.89	153.27	157.00	100.53	149.55	-	83.56	84.01	31.89	26.03	81.47	64.01	115.07
81	124.73	152.76	158.05	100.91	150.37	-	110.73	83.56	31.64	26.41	81.30	63.49	166.41

E. Experimental Section

General Procedures

Melting points were determined on a Reichert microstage apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker WH-200 or Bruker WH-400 spectrometers operating in the FT mode, with Me_4Si as internal reference in Me_2SO-$\underline{d}_6$ unless specified otherwise. Ultraviolet (UV) spectra were recorded on a Cary 15 spectrophotometer and infrared (IR) spectra on a Nicolet 7199 FT(IR) instrument. Optical rotations were determined using a Perkin-Elmer Model 141 polarimeter with a 10-cm 1-mL microcell. Mass Spectra (MS) were determined by the mass spectrometry laboratory of this department on an AEI MS-50 instrument with computer processing at 70eV using a direct probe for sample introduction. Elemental analyses were determined by the Microanalytical Laboratory of this department or by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. Evaporations were effected using a Buchler rotating evaporator equipped with a Dewar "dry-ice" condenser under water aspirator or mechanical oil pump vacuum at 30°C or cooler. Thin layer chromatography (TLC) was performed on E. Merck chromatograph sheets (silica gel 60 F_{254}, layer thickness 0.2 mm, catalogue 5775) with sample observation under UV light (2537 Å). Preparative TLC was

performed on glass plates coated with Merck silica PF 254. The solvents used for TLC were different ratios of methanol-chloroform (1:50, 1:20, 1:10) and the upper phase of EtOAc-nPrOH-H_2O (4:1:2). Silica gel column chromatography was performed using Mallinckrodt CC-7 (200 mesh) silica gel. Anion exchange chromatography was carried out on Dowex 1x2 resin in the hydroxide form.

All solvents used were of reagent grade and were distilled prior to use. Purification of most solvents and reagents was accomplished according to methods described in reference 125. All dried solvents were stored over Davison 4A molecular sieves purchased from the Fisher Scientific Company.

For conversion of ribonuclesides to their bromo acetate derivatives, General Method A is described in detail for adenosine and General Method B for inosine.

General Method C describes the reductive elimination of bromo acetate mixtures and deprotection to give the 2',3'-unsaturated nuclesides. It is described in detail for the preparation of 9-(2,3-dideoxy-β-D-glycero-pent-2-eno-furanosyl)adenine (37).

α-Acetoxyisobutyryl Chloride (61a)

To 50g (48 mmol) of α-hydroxyisobutyric acid at 0°C was added slowly with stirring 90.5 mL (1.27 mole) of freshly distilled acetyl chloride. The solution was stirred for one hour at 0°C and the resulting brown colored solution was then allowed to warm to room temperature. Stirring was continued until evolution of hydrogen chloride gas ceased and the solution was then refluxed for two hours. Excess acetyl chloride was removed by evaporation and 30.7 mL (0.421 mole) of freshly distilled thionyl chloride was added dropwise with stirring. This mixture was heated at 80°C for 5 h. The resulting solution was evaporated and the product distilled to give 63.9 g of (61a): bp 35-37°C/2mm of Hg; n_D^{24} 1.4286 (lit[105] bp 55-56°C/6 mm of Hg; n_D^{25} 1.4278)

α-Acetoxyisobutyryl Bromide (61b)

To an oven-dried 1000 mL 3-necked round bottom flask was added 64.1 g of LiBr (0.745 mole, dried for 72 h at 110°C) and 400 mL of dry (twice distilled from P_2O_5) EtOAc. This stirred suspension was heated at reflux for 1 h while protected from moisture (when the LiBr and EtOAc are dry, a yellow solution results). Dropwise (30 min) addition of 82 g (0.498 mole) of (61a) and continued heating of the resulting mixture at reflux for 1 h was followed by evaporation of solvent. The resulting yellow oil (with a heavy white ppt of LiCl) was distilled to give 95.5 g (92%) of (61b): bp 55-56°C/5 mm Hg; n_D^{25} 1.4566; ^1H nmr δ ($CDCl_3$) 1.57 (s, 6, CMe_2); 2.10 (s, 3, OAc) (lit[105] bp 75-77°C/12 mm Hg; n_D^{25} 1.4530)

(Zinc/copper couple) (Quantity used for 1 mmol of nucleoside)

To a suspension of 2 g zinc dust in 20 mL of water was added a concentrated aqueous solution of 0.4 g of $CuSO_4.5H_20$. The suspension was stirred vigorously for 10 min, the precipitate was filtered, washed with water, washed with dimethylformamide and immediately used in the reductive elimination step. This preparation should be kept wet and not allowed to stand.

General Method A. is illustrated by the preparation of 9-(2-O-Acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl) and 9-(3-O-Acetyl-2-bromo-2-deoxy-β-D-arabinofuranosyl)adenine and their 5'-O-(2,5,5-Trimethyldioxolan-4-on-2-yl) derivatives (67c),(68c),(67b),(68b), respectively.

To 267 mg (1 mmol) of dried adenosine (6) was added 20 mL of dry CH_3CN, 2 mL of CH_3CN/H_2O (100:1) and 600 μL (4 mmol) of 61b. The resulting suspension was stirred at room temperature for 1 h (the reaction mixture became a clear solution after approximately 45 min and at approximately 50 min a fine precipitate began to separate). Saturated $NaHCO_3/H_2O$ (25 mL) was added and the solution was extracted with 2x50 mL of EtOAc. The organic phase was washed with 20 mL of saturated $NaCl/H_2O$, dried (Na_2SO_4), and evaporated to yield a colourless crude foam.

UV spectral analysis of the combined aqueous phase showed approximately 3 mg of adenine (1) (2% cleavage). ^1H nmr spectra of the obtained compounds were in agreement with literature values.[106]

General Method B. is illustrated in the first paragraph of the following preparation of 9-(2,3-Anhydro-β-D-ribofuranosyl)hypoxanthine (88).

To 268 mg (1 mmol) of dried inosine (72) was added 20 mL of dry CH_3CN and 1.2 mL (8 mmol) of (61b). The resulting mixture was allowed to stand at room temperature for 3 h (a clear solution resulted after 15 min and remained so throughout the reaction period). Saturated $NaHCO_3/H_2O$ (40 mL) was added and the solution was extracted with 2x50 mL portions of EtOAc. The organic phase was washed with 20 mL of saturated $NaCl/H_2O$), dried (Na_2SO_4), and evaporated to give an almost colourless foam.

UV analysis of the combined aqueous phase showed 6 mg of hypoxanthine (4% cleavage).

The crude foam was stirred with 25 mL of anion exchange resin (IRA-400, OH^-, previously washed with absolute MeOH) at room temperature for 1 h. The resin was filtered and washed with MeOH. Elution of product from the resin was effected with 30 mM aqueous triethylammonium bicarbonate solution. The off-white compound obtained after evaporation (<30°C) of the combined solutions was purified by chromatography (silica gel, 2x20 cm, $CHCl_3$-MeOH, 97:3, v/v) and crystallized from EtOH to give 202 mg (81%) of (88): mp 210°C (dec) (lit[120] mp 226-230°C); uv (MeOH) max 249 nm (ε 12,700); M.S. m/z 250.0702, Calc. for M 250.0697; ^1H nmr δ 3.55 (m, 2, H5',5"), 4.20 ("t", 1, H4'), 4.30 (d, 1, H3'),

4.51 (d, 1, H2'), 5.10 (bs, 1, OH5'), 6.25 (s, 1, H1'), 8.04 (s, 1, H2), 8.06 (s, 1, H8); Anal. Calc. for $C_{10}H_{10}N_4O_4$: C 48.00, H 4.03, N 22.39. Found: C 48.12, H 4.07, N 22.26.

9-(2,3-Anhydro-β-D-ribofuranosyl)adenine (47)

A 1 mmol sample of (6) was treated by Method A. The crude foam was dissolved in 10 mL of absolute MeOH and treated with 25 mL of anion exchange resin (IRA-400, OH^-, previously washed with absolute MeOH). The resulting suspension was stirred at room temperature for 1 h. The resin was filtered and washed with MeOH. Evaporation of the combined MeOH fractions yielded an almost colourless solid that was crystallized from EtOH to give 0.23 g (92%) of powdery (47).

A larger scale experiment using 66 mmol of (6) gave 47 in 93% yield: mp 180-181 (dec) (lit[100] mp 180°C); uv (MeOH) max 258 nm (ε 14,600); M.S. m/z 249.0858, Calc. for M 249.0862; ^{1}H nmr δ 3.54 (m, 2, H5',5"), 4.18 ("t", 1, H4'), 4.22 (d, 1, H3'), 4.46 (d, 1, H2'), 5.05 (bs, 1, OH5'), 6.21 (s, 1, H1'), 7.33 (bs, 2, NH_2), 8.18 (s, 1, H2), 8.34 (s, 1, H8); Anal. Calc. for $C_{10}H_{11}N_5O_3$: C 48.19, H 4.45, N 28.10. Found: C 48.07, H 4.46, N 28.01.

2'-Deoxyadenosine (10) and 3'-Deoxyadenosine (73)

The crude foam obtained from a 2 mmol reaction of (6) following Method A was dissolved in 40 mL of oxygen free toluene. After addition of 1.21 g (4 mmol) n-Bu_3SnH and 32 mg (0.2 mmol) AIBN, the mixture was refluxed for 3 h. The oily residue obtained after evaporation was treated with saturated NH_3/MeOH (20 mL) for 14 h. The syrupy residue was partitioned between 20 mL of water and 20 mL of ether. The aqueous layer was concentrated (approximately 5 mL) and applied to a Dowex 1x2 (OH^-) column (2x20 cm). Elution with water, evaporation and crystallization of the residue from EtOH gave 51 mg (10.2%) of 10: mp 191-192°C (lit'°° mp 190-192°C); uv (H_2O) max 260 nm (ϵ 15,200); M.S. m.z 251.1017, calc. for M 251.1018; ^1H nmr δ 2.01, 2.39 (m, 2, H2',2"), 3.60 (m, 2, H5',5"), 3.93 (m, 1, H4'), 4.45 (m, 1, H3'), 5.30 (dd, 1, OH5'), 5.36 (d, 1, OH3'), 6.38 (dd, 1, H1'), 7.34 (bs, 2, NH_2), 8.15 (s, 1, H2), 8.26 (s, 1, H8); Anal. Calc. for $C_{10}H_{13}N_5O_3$: C 47.81, H 5.21, N 27.87. Found: C 47.53, H 5.20, N 27.88.

Further elution of the column with MeOH/H_2O (3:7, v/v), evaporation and crystallization of the residue from EtOH gave 399 mg (79.5%). of 73: mp 224-225°C (lit'°° mp 228-229°C); uv (H_2O) max 260 nm (ϵ 14,900); M.S. m.z 251.1015, Calc. for M 251.1018; ^1H nmr δ 1.93 (ddd, 1, H3'), 2.28 (ddd, 1, H3"), 3.61 (m, 2, H5',5"), 4.37 (m, 1, H4'), 4.59 (m, 1, H2'), 5.16 (dd, 1, OH5'), 5.66 (d, 1, OH2'),

5.85 (d, 1, H1'), 7.25 (bs, 2, NH_2), 8.17 (s, 1, H2), 8.35 (s, 1, H8); Anal. Calc. for $C_{10}H_{13}N_5O_3$: C 47.81, H 5.21, N 27.87. Found: C 47.67, H 5.20, N 27.80.

General Method C is illustrated by the preparation of 9-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)adenine (37)

The foam obtained from a 2 mmol reaction of (6) following Method A was dissolved in 5 mL of pyridine and treated with 540 mg (5 mmol) of freshly distilled acetic anhydride and 12 mg (0.1 eqiv.) of 4-dimethylaminopyridine (DMAP). This solution was stirred for 10 h at room temperature, evaporated and the residue co-evaporated with toluene. The resulting yellow foam was dissolved in 25 mL of DMF and treated with freshly prepared zinc-copper couple at room temperature for 1 h. The suspension was filtered using celite, washed with DMF and evaporated (<30°C) to give a yellow foam that was treated with 10 mL of saturated NH_3/MeOH at room temperature for 10 h.

Following evaporation, the residue was dissolved in H_2O and applied to a column of Dowex 1x2 (OH^-). Elution with H_2O, evaporation and crystallization of the residue from methanol with diffusion of ether gave 377 mg (81%) of 37: mp 194-196°C (lit[10] mp 194-195°C); uv (MeOH) max 259 nm (ε 14,900); M.S. m/z 233.0915, Calc. for M 233.0912; ^{1}H nmr δ 3.64 (dd, 2, H5',5"), 4.92 (m, 1, H4'), 5.11 ("t", 1, OH5'), 6.16 (ddd, 1, H2'), 6.48 (ddd, 1, H3'), 6.98 (ddd, 1, H1'), 7.28 (bs, 2, NH_2), 8.18 (s, 1, H2), 8.20 (s, 1, H8); Anal. Calc. for $C_{10}H_{11}N_5O_2$: C 51.59, H 4.75, N 30.03. Found: C 51.35, H 4.75, N 30.03.

9-(2,3-Dideoxy-β-D-glycero-pentofuranosyl)adenine (89)

A solution of 233.3 mg (1 mmol) of (37) in 10 mL of 98% EtOH was hydrogenated at 10 psi on a Parr shaker in the presence of 100 mg of 5% Pd/C at room temperature for 5 h. Filtration, evaporation of the filtrate and recrystallization of the residue from absolute EtOH gave 212 mg (89%) of (89): mp 185-187°C (lit[12] mp 187-188°C); M.S. m/z 235.1067, Calc. for M 235.1069; ^{1}H nmr δ 2.18 (m, 2, H3',3"), 2.39 (m, 2, H2',2"), 3.56 (dd, 2, H5',5"), 4.06 (m, 1, H4'), 4.93 ("t", 1, OH5'), 6.08 (dd, 1, H1'), 8.13 (s, 1, H2), 8.30 (s, 1, H8). Anal. Calc. for $C_{10}H_{13}N_5O_2$: C 51.06, H 5.57, N 29.77. Found: C 50.98, H 5.56, N 29.74.

9-(3-Deuterio-2,3-dideoxy-β-D-glycero-pent-2-eno-furanosyl)adenine (87)

A 67 mg (0.25 mmol) sample of 3'-deuterioadenosine (86) was treated by General Methods A and C to give 44.5 mg (76%) of (87): mp 193-194°C; M.S. m/z 234.0974, Calc. for M 234.0976; ^{1}H nmr δ 3.60 (d, 2, H5',5"), 4.90 (m, 1, H4'), 5.07 ("t", 1, OH5'), 6.14 (dd, 1, H2'), 6.95 (dd, 1, H1'), 7.27 (bs, 2, NH_2), 8.15 (s, 1, H2), 8.17 (s, 1, H8).

9-(3-Deuterio-2,3-dideoxy-β-D-glycero-pentofuranosyl)-adenine (90)

A 15 mg (0.06 mmol) sample of (87) was hydrogenated under the identical conditions described for conversion of (37) to (89), to give 12 mg (80%) of (90): M.S. m/z 236.1135, Calc. for 236.1132; ^{1}H nmr δ 2.01 (m, 1, H3'), 2.36 (m, 2, H2',2"), 3.46, 3.61 (2xdd, 2, H5',5"), 4.11 (m, 1, H4'), 5.04("t", 1, OH5'), 6.19 (dd, 1, H1'), 8.13 (s, 1, H2), 8.34 (s, 1, H8).

9-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)hypoxanthine (82)

The crude foam from treatment of 1 mmol of (72) by General Method B was dissolved in 5 mL of dry pyridine and stirred with 270 mg (2.5 mmol) of acetic anhydride and 12 mg (0.1 eq.) of 4-dimethylaminopyridine (DMAP) at room temperature for 10 h. The solution was evaporated and the residue co-evaporated twice with toluene to give a yellow foam. This was dissolved in 10 mL of DMF and treated with freshly prepared zinc/copper couple at room temperature for 1 h. The suspension was filtered using celite and the filter cake was washed with DMF. The filtrate was evaporated and the resulting yellow syrup was dissolved in MeOH and stirred with 15 mL of anion exchange resin (Dowex 1x2, OH^-, previously washed with MeOH) at room temperature for 20 min. The resin was filtered and washed with MeOH. Elution of product was accomplished by washing the resin with 30 mM triethylammonium bicarbonate (150 mL). Evaporation of the combined washes gave a slightly yellow solid that was purified by chromatography (silica gel, 2x20 cm, MeOH/$CHCl_3$, 3:97, v/v). Evaporation of the appropriate fractions and crystallization of the residue from methanol with diffusion of ether gave 164 mg (70%) of (82): M.S. m/z 234.0757, Calc. for M 234.0753; ^{1}H nmr δ 3.55 (d, 2, H5',5"), 4.85 (m, 1, H4'), 5.10 ("t", 1, OH5'), 6.11 (ddd, 1, H2'), 6.43 (ddd, 1, H3'), 6.75 (ddd, 1, H1'), 8.04 (s, 1, H2), 8.08 (s, 1, H8);

Anal. Calc. for $C_{10}H_{10}N_4O_3$: C 51.28, H 4.30, N 23.92. Found: C 51.02, H 4.31, N 23.86.

4-Amino-7-(2,3-anhydro-β-D-ribofuranosyl)pyrrolo[2,3-d]-pyrimidine (77)

A 266 mg (1 mmol) sample of tubercidin (71) was treated by the conditions described for the preparation of 9-(2,3-anhydro-β-D-ribofuranosyl)adenine (47). Recrystallization of the product from 95% EtOH with diffusion of ether gave 255 mg (91%) of 77: mp 170-172°C (lit[67] mp 170-173°C); uv (MeOH) max 271 nm (ε 12,100); M.S. m/z 248.0911, Calc. for M 248.0909; ^{1}H nmr δ 3.52 (m, 2, H5',5"), 4.11 ("t", 1, H4'), 4.16 (d, 1, H3'), 4.25 (d, 1, H2), 5.05 (bs, 1, OH5'), 6.25 (s, 1, H1'), 6.60 (d, 1, H5), 7.02 (bs, 2, NH_2), 7.30 (d, 1, H6), 8.08 (s, 1, H2); Anal. Calc. for $C_{11}H_{12}N_4O_3$: C 53.22, H 4.86, N 22.57. Found: C 53.02, H 4.80, N 22.51.

4-Amino-7-(2,3-dideoxy-β-D-glycero-pent-2-eno-furanosyl)-pyrrolo[2,3-d]pyrimidine (77)

A 266 mg (1 mmol) sample of tubercidin was treated by General Methods A and C. Crystallization of the product from methanol with diffusion of ether gave 209 mg (90%) of 77: mp 210-211°C (lit[10] mp 208-210°C); uv (EtOH) max 270 nm (ε 12,000); M.S. m/z 232.0965, Cac. for M 232.0960; ^{1}H nmr δ 3.54 ("t", 2, H5',5"), 4.79 (m, 1, H4'), 4.97 ("t", 1, H5'), 6.03 (ddd, 1, H2'), 6.43 (ddd, 1, H3'), 6.58 (d, 1, H5), 7.02, (bs, 2, NH_2), 7.12 (ddd, 1, H1'), 7.17 (d, 1, H6), 8.08 (s, 1, H2); Anal. Calc. for $C_{11}H_{12}N_4O_2$: C 56.89, H 5.21, N 24.13. Found: C 56.74, H 5.29, N 24.04.

4-Amino-5-cyano-7-(2,3-dideoxy-β-D-glycero-pent-2-eno-furanosy)pyrrolo[2,3-d]pyrimidine (78)

A 291 mg (1 mmol) sample of toyocamycin (75) was subjected to General Method A and the crude product was treated by General Method C to the end of the first paragraph. The crude product obtained after deprotection with saturated NH_3/MeOH and evaporation was purified by column chromatography (silica gel, 2x20 cm, MeOH/$CHCl_3$, 3:97, v/v) and crystallized from ethanol with diffusion of ether to give 206 mg (81%) of (78) as a slightly off-white powder: mp 165-166°C; uv (MeOH) max 278 nm (ε 15,300); M.S. m/z 257.0915, Calc. for M 257.0913; ^{1}H nmr δ 3.59 (dd, 2, H5',5"), 4.89 (m, 1, H4'), 5.02 ("t", 1, OH5'), 6.08 (ddd, 1, H2'), 6.48 (ddd, 1, H3'), 6.88 (s, 2, NH_2), 7.13 (ddd, 1, H1'), 8.22 (s, 1, H2), 8.25 (s, 1, H6); Anal. Calc. for $C_{12}H_{11}N_5O_2$: C 56.03, H 4.31, N 27.22. Found: C 56.17, H 4.29, N 27.06.

4-Amino-5-cyano-7-(2,3-dideoxy-β-D-glycero-pentofuranosyl)-pyrrolo[2,3-d]pyrimidine (80)

A 129 mg (0.5 mmol) sample of (78) was hydrogenated as described for the conversion of (37) to (89). Crystallization of the product from absolute ethanol with diffusion of ether gave 115 mg (89%) of (80): mp 171-172 °C; uv (MeOH) max 278 nm (ε 15,600); M.S. m/z 259.1063, Calc. for M 259.1069; ^{1}H nmr δ 2.20 (m, 2, H3',3"), 2.40 (m, 2, H2',2"), 3.58 (dd, 2, H5',5"), 4.05 (m, 1, H4'), 5.04("t", 1, OH5'), 6.84 (bs, 2, NH_2), 6.96 ("t", 1, H1'), 8.22(s, 1, H2), 8.24 (s, 1, H6); Anal. Calc. for $C_{12}H_{13}N_5O_2$: C 55.59, H 5.05, N 27.01. Found: C 55.36, H 5.09, N 26.84.

4-Amino-5-carboxamido-7-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)pyrrolo[2,3-d]pyrimidine (79)

A 309 mg (1 mmol) sample of sangivamycin (76) was subjected to General Methods A and C. Crystallization of the product from methanol with diffusion of ether gave 220 mg (80%) of (79) as a slightly off-white powder: mp 185-186°C; uv (MeOH) max 278 nm (ε 12,500); M.S. m/z 275.1020, Calc. for M 275.1018; 'H nmr δ 3.50 (dd, 2, H5',5"), 4.78 (m, 1, H4'), 4.91 ("t", 1, OH5'), 6.31 (ddd, 1, H2'), 6.57 (ddd, 1, H3'), 7.10 (ddd, 1, H1'), 7.30 (s, 2, NH_2), 7.96 (s, 1, H2), 8.11 (s, 1, H6); Anal. Calc. for $C_{12}H_{13}N_5O_3$ C 52.36, H 4.76, N 25.44. Found: C 52.09, H 4.75, N 25.26.

4-Amino-5-carboxamido-7-(2,3-dideoxy-β-D-glycero-pento-furanosyl)pyrrolo[2,3-d]pyrimidine (81)

A 138 mg (0.5 mmol) sample of (79) was hydrogenated as described for the conversion of (37) to (89). Crystallization of the product from absolute ethanol with diffusion of ether gave 127.5 mg (92%) of (81): mp 205-206°C; uv (MeOH) max 279 nm (ε 12,700);) M.S. m/z 277.1177, Calc. for M 277.1175; ^{1}H nmr δ 2.25 (m, 2, H3',3"), 2.43 (m, 2, H2',2"), 3.54 (dd, 2, H5',5"), 4.07 (m, 1, H4'), 4.86 ("t", 1, OH5'), 6.38 ("t", 1, H1'), 7.32 (bs, 2, NH_2), 7.96 (bs, 2, NH_2), 8.08 (s, 1, H2), 8.10 (s, 1, H6); Anal. Calc. for $C_{12}H_{15}N_5O_3$: C 51.98, H 5.45, N 25.26. Found C 51.75, H 5.41, N 25.11.

1-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)cytosine (85)

A 285 mg (1 mmol) sample of 4-N-acetylcytidine (84) was treated by General Methods A and C. Crystallization of the product from methanol with diffusion of ether gave 294 mg (70%) of (85) mp 162-163°C (lit[103] mp 168-169°C); uv (H_2O) max 271 nm (ε 8,800); M.S. m/z 210.0884, Calc. for M 210.0879; 'H nmr δ 3.56 (d, 2, H5',5"), 4.76 (m, 1, H4'), 4.98 (bs, 1, OH5'), 5.79 (d, 1, H5), 5.88 (ddd, 1, H2'), 6.34 (ddd, 1, H3'), 6.99 (ddd, 1, H1'), 7.19 (bs, 2, NH_2), 7.71 (d, 1, H6); Anal. Calc. for $C_9H_{12}N_3O_3$: C 51.42, H 5.75, N 19.99. Found: C 51.40, H 5.73, N 19.91.

1-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)uracil (34)

To 488 mg (2 mmol) of uridine (9) was added 10 mL of dry CH_3CN and 800 μL of α-acetoxyisobutyryl bromide (61b). The resulting solution was heated at 80°C for 3 h. Saturated $NaHCO_3/H_2O$ (30 mL) was added and the solution was extracted with 3x50 mL of EtOAc. The organic phase was washed with 20 mL of saturated $NaCl/H_2O$, dried (Na_2SO_4) and evaporated to yield a colourless foam. This was dissolved in 15 mL of $HOAc/H_2O$ (1:1) cooled to -15°C and treated with a solution of 13 g of $NaOAc.3H_2O$ in 13 mL of $HOAc/H_2O$ (1:1) (previously cooled to -15°C). Freshly prepared zinc/copper couple (H_2O washed) was added and the suspension was stirred for 1 h at -15°C. Solid $NaHCO_3$ was added slowly at 0°C to neutrality. The suspension was filtered using celite and the filter cake was washed with 20 mL EtOAc. The aqueous layer was extracted with 2x50 mL of EtOAc. The combined organic phase was washed with 20 mL of saturated $NaCl/H_2O$, dried (Na_2SO_4) and evaporated. The crude product was treated with NH_3/MeOH (15 mL) at room temperature for 10 h. The solution was evaporated and the residue was crystallized from ethanol with diffusion of ether to give 208 mg (50%) of (34): mp 156-158°C (lit[10] mp 154.5-155.5°C); uv (H_2O) max 261 nm (ε 9700); M.S. m/z 210.0637, Calc. for M 210.0641; ^1H nmr δ 3.59 (d, 2, H5',5"), 4.75 (m, 1, H4'), 5.02 ("t", 1, OH5'), 5.67 (d, 1, H5), 5.93 (ddd, 1, H2'), 6.40 (ddd, 1, H3'), 6.78 (ddd, 1, H1'), 7.73 (d, 1, H6), 11.53 (bs, 1, H3);

Anal. Calc. for $C_9H_{11}N_2O_4$: C 57.42, H 4.80, N 13.33. Found: C 57.19, H 4.78, N 13.22.

9-(2,3-Dideoxy-β-D-glycero-pent-2-enofuranosyl)guanine (83)

A 283 mg (1 mmol) sample of guanosine (7) was treated by General Method B. In this case, a suspension remained throughout the reaction period. The crude foam obtained was subjected to the conditions used for the preparation of 9-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)hypoxanthine (82). Crystallization of the product from methanol with diffusion of ether gave 117 mg (47%) of (83): mp >300°C; uv (H_2O) max 253 nm (ε 14,000); M.S. m/z 249.0852, Calc. for M 249.0862; 'H nmr δ 3.54 (d, 2, H5',5"), 4.83 (m, 1, H4'), 4.97 ("t", 1, OH5'), 6.09 (ddd, 1, H2'), 6.44 (ddd, 1, H3'), 6.50 (bs, 2, NH_2), 6.70 (m, 1, H1'), 7.72 (s, 1, H8), 10.66 (bs, 1, NH); Anal. Calc. for $C_{10}H_{11}N_5O_3$: C 48.19, H 4.45, N 28.10. Found: C 47.76, H 4.41, N 27.58.

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